California Air Resources Board Chairman's Air Pollution Seminar Series

INTERDISCIPLINARY AIR QUALITY RESEARCH at the UNIVERSITY OF CALIFORNIA, DAVIS

April 13, 2006

Sacramento, California

Air Resources Board

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Air Quality Research Center (AQRC)

Anthony Wexler, Director

and

San Joaquin Valley Health Effects Research Center (SAHERC)

Anthony Wexler, Director

Kent Pinkerton, co-Director

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Air Pollution and Health: Interdisciplinary, or what?

"Impact of community air pollution on health is one of the most complicated health problems of the day because of:

- the extensive range of scientific disciplines needed to describe the essential elements of the problems as well as
- the interaction of subjective and objective effects in need of investigation."

"Not only are all the **medical sciences** and specialties required to describe the health effects of pollution, but **meteorology, chemistry, physics, sociology, mathematics, psychology, and statistics** are involved. "

M.H. Merrill, Director

California State Department of Public Health, 1964

Air Quality Research Center - Summary Statistics

http://airquality.ucdavis.edu

- **Over 60 faculty in science, engineering and policy**
  - **Across four colleges and Schools of Medicine and Veterinary Medicine**
  - **Individual faculty have over $40M in funding**
  - **Agricultural Emission Center, Frank Mitloehner**

- **Current Funding**
  - **EPA / SAHERC $8M**
  - **AAH / UCOP $1.6M**

- **Pending or Proposals Under Development**

  Health Effects Institute -- $4M
  Ag Emissions -- ~$15M
  NIH/DISCOVER -- $10M

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Air Quality and Health: The Basic Problem

- Epidemiological studies show association between particulate matter and increased morbidity and mortality
- What is it about particles that cause health effects?
- What health effects do they cause?

San Joaquin Valley Aerosol Health Effects Research Center (SAHERC): Overview

Particle Sampling

- Particulates collected in SJV
- Analyzed for size, chemical comp and number
- Synthetic ambient particles generated in laboratory
- Why? Control composition and size for health research

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SAHERC Overview (cont.)

Health Effects

- Metabolic response of pulmonary and cardiovascular cells to particles
- Toxicological response: particle size, shape or composition
- Mapping how particles move from the lungs to other organs
- How particles affect lung development during childhood

SAHERC Projects and Cores

- **Projects**
  - 1: Pulmonary Metabolic Response
  - 2: Cardiovascular Metabolic Response
  - 3: SJV Aerosol Inhalation Exposure
  - 4: Transport and Fate of Particles
  - 5: Architecture Development
- **Cores**
  - Animal Exposure
  - Particle Generation, Modification, and Characterization
  - San Joaquin Valley
  - Imaging
  - Bioanalytical
  - Quality Management
  - Administrative

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1. Pulmonary Metabolic Response

Michelle Fanucchi, Charles Plopper,
Alan Buckpitt

Prior Epidemiological studies

- Postnatal lung development has been demonstrated to be a critical period of susceptibility to air pollution
- Increased morbidity and mortality associated with infant exposure to environmental particulate air pollution

Project Objective

- Determine the impacts of individual components of particulate matter, (including PAHs and transitional metals) both separately and as mixtures
- Examine the effects on cytotoxicity, protein sulfhydryl oxidation status, and early gene expression alterations in postnatal and adult airways of both healthy and oxidative stressed individuals

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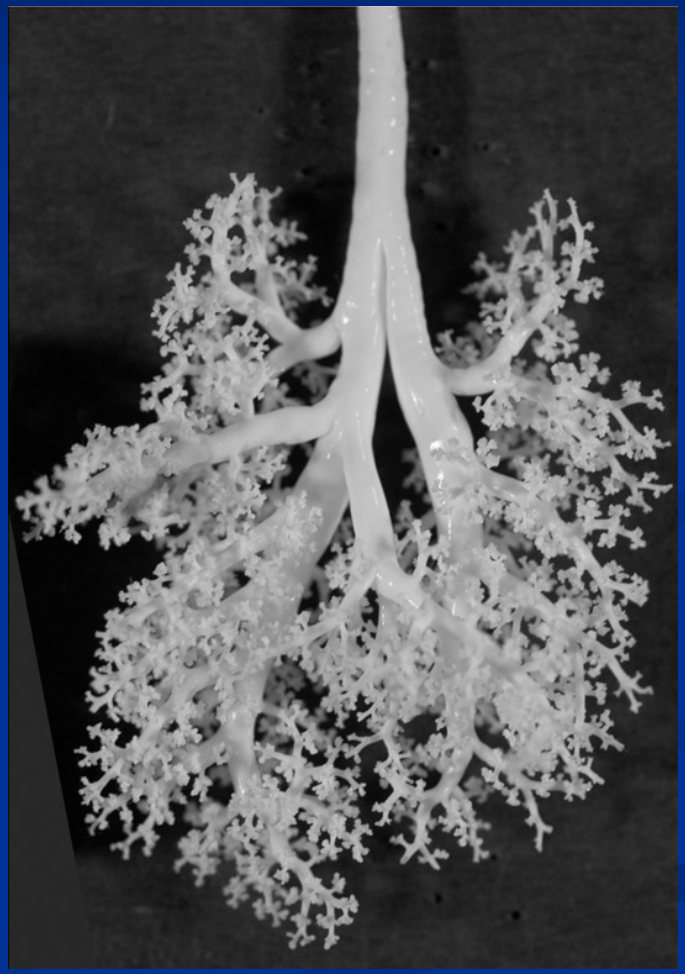
Critical Events During Lung Development

- Overall growth
- Branching morphogenesis
- Cellular proliferation
- Cellular differentiation
- Matrix formation



Issues for Neonatal Susceptibility

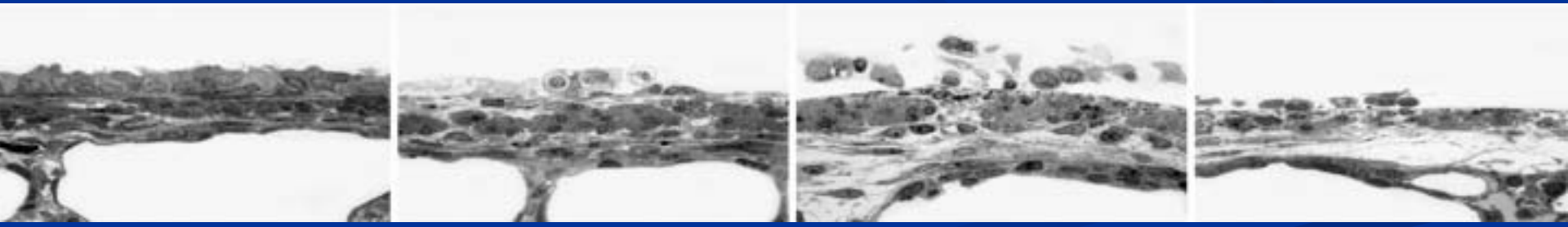
- Acute cytotoxicity
- Repair following injury
- Atypical lung development

Acute Cytotoxicity: 1-Nitronaphthalene

Adults

Control | 12.5 mg/kg | 50 mg/kg | 100 mg/kg

Neonates



Control | 12.5 mg/kg | 50 mg/kg | 100 mg/kg

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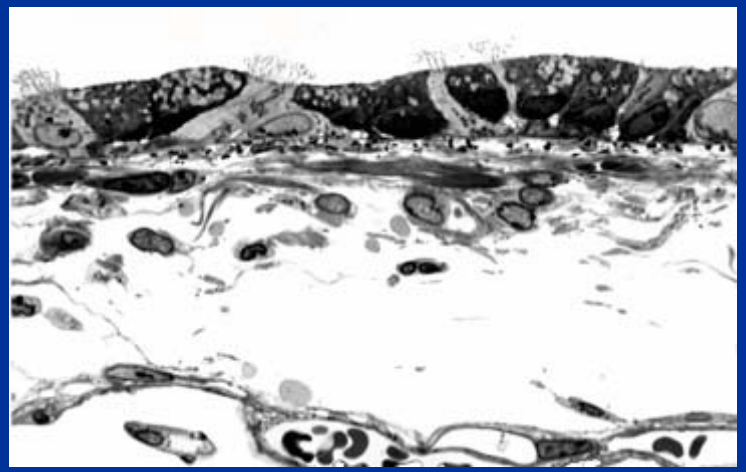
Repair Following Injury: Naphthalene

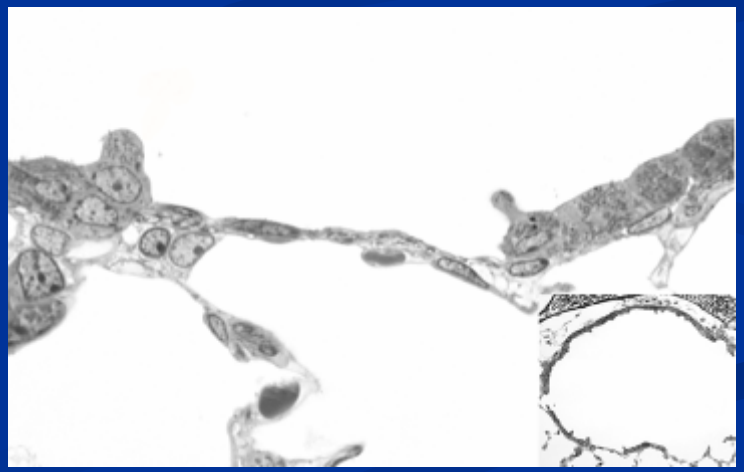
Lobar Bronchus

Terminal Bronchiole

6 Week Old Carrier Treated Mouse



Lobar Bronchus



Terminal Bronchiole

6 Week Old NA Treated Mouse

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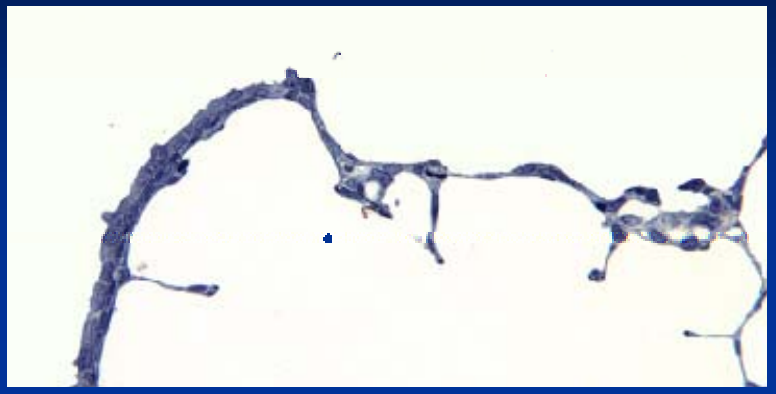
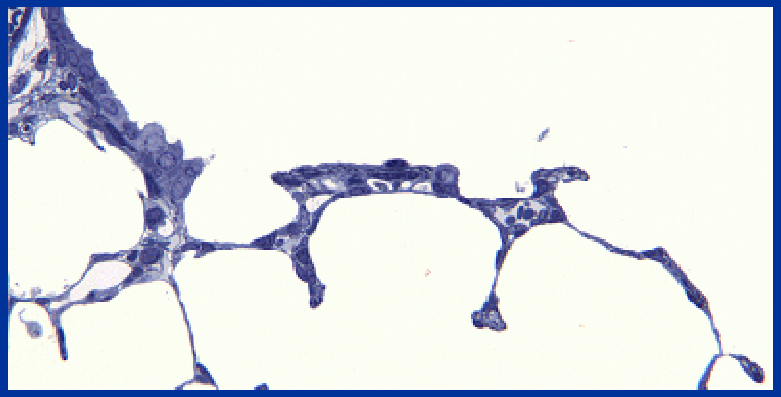
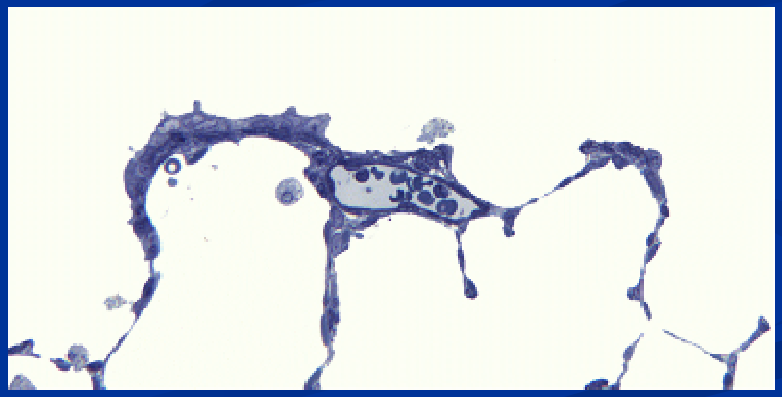
Repair Following Injury: 1-Nitronaphthalene



Terminal Bronchiole

21 Day Old Carrier-Treated Rat





Terminal Bronchioles

21 Day Old 1NN-Treated Rats

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1. Pulmonary Metabolic Response

Hypothesis

- *The differentiating epithelium of the neonatal lung is more susceptible to particulate matter-induced pulmonary injury than the differentiated epithelium of the adult lung*

1. Pulmonary Metabolic Response

Strategy

Use defined synthetic particles to understand the role that particle composition plays in the acute injury of the neonatal lung.

1. Pulmonary Metabolic Response

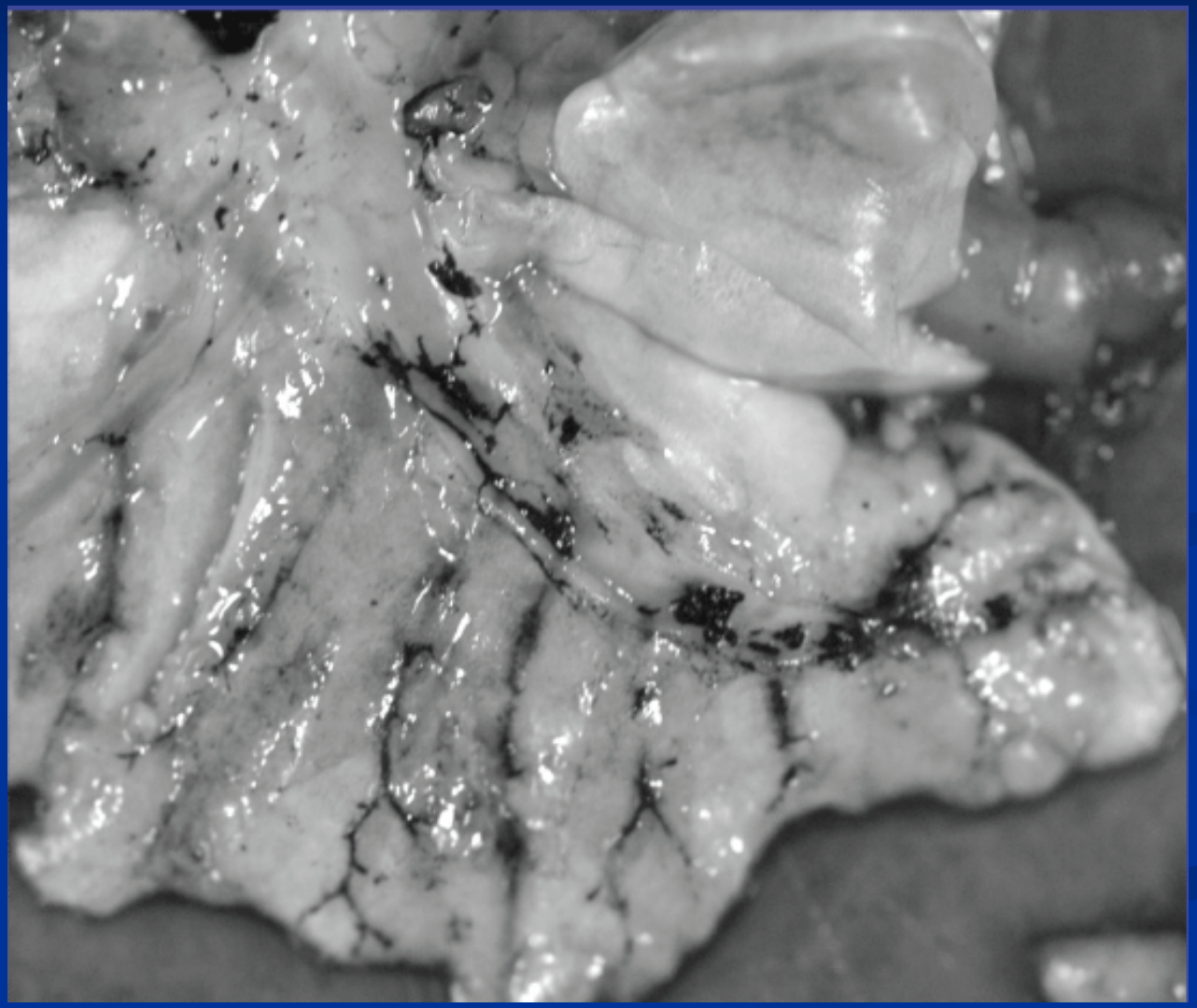
Specific Aim 1

- Compare the pulmonary cytotoxic response of a PAH alone and adhered to a particle in postnatal and adult rats.

Approach

- Synthetic particle: carbon + PAH (*currently 1-nitronaphthalene*)
- Exposure: Single intratracheal insufflation: 7-day and adult rats
- Evaluation: Early and late timepoints
  - initial epithelial injury
  - particle clearance
  - site-specific proliferation
  - gene expression profiles
  - site-specific oxidative stress

Initial Particle Distribution

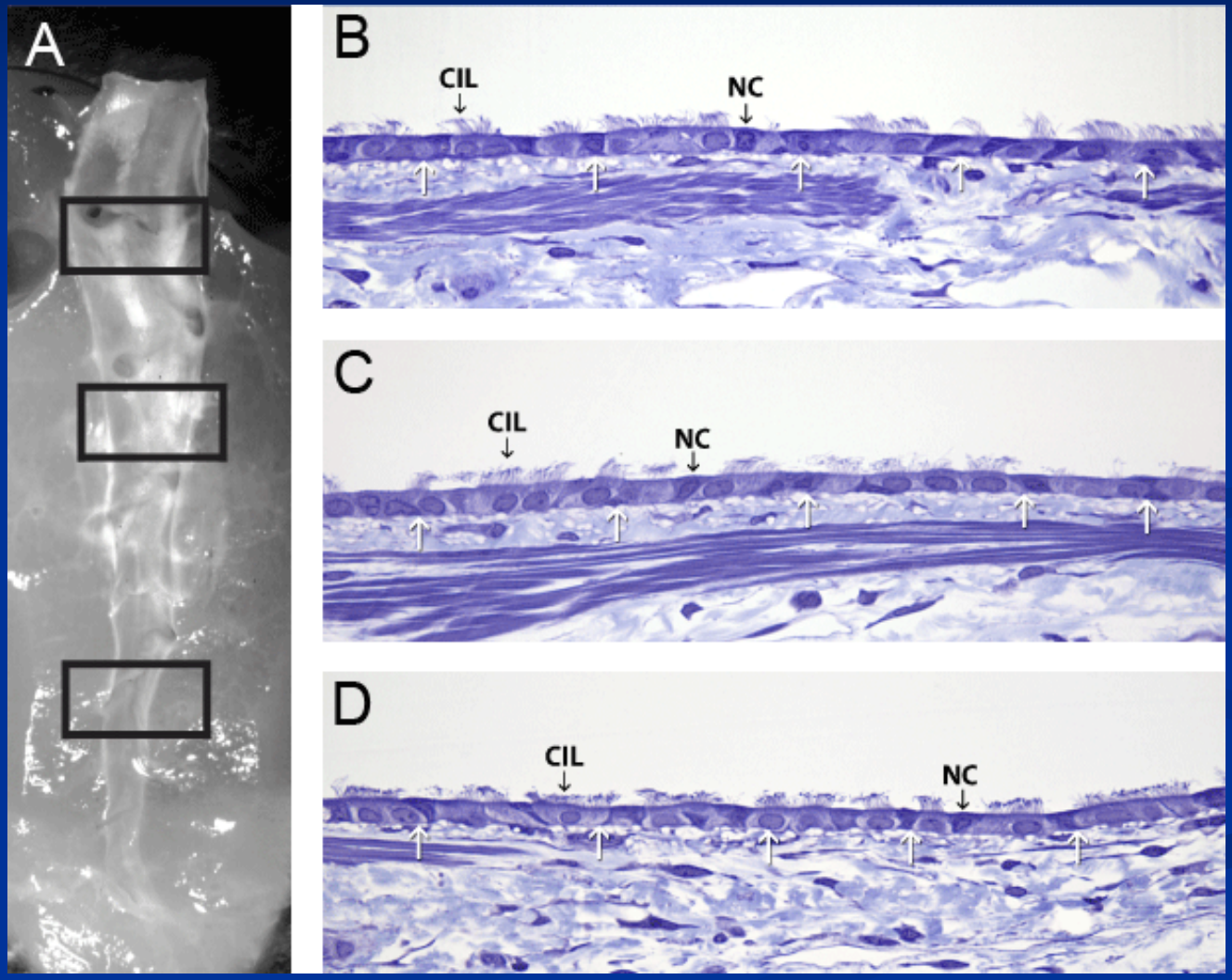


Uncoated Carbon Particles – 2 hours post insufflation

Generation 2

Generation 5

Generation 11

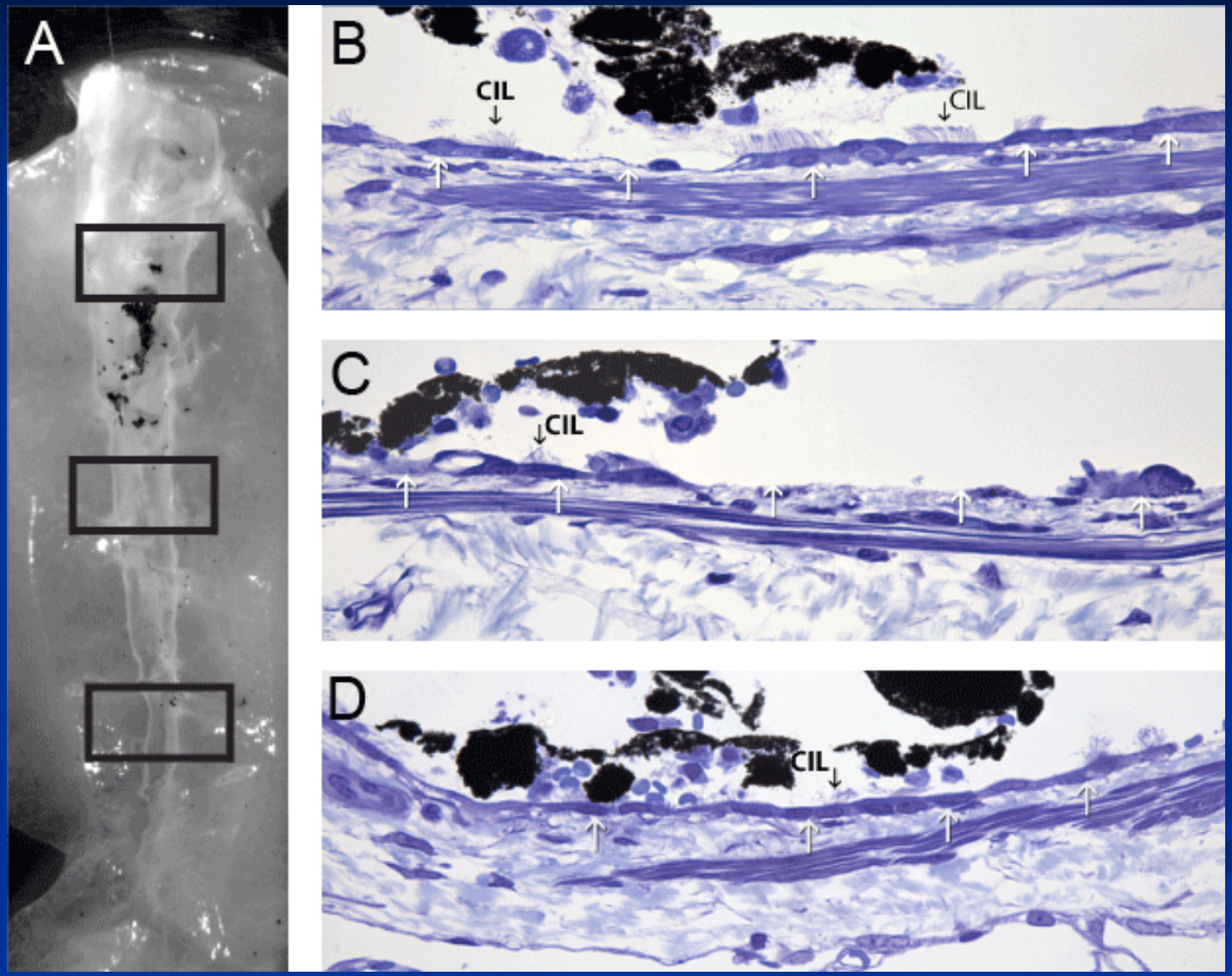


10% 1-NN Carbon Particles – 2 hours post insufflation

Generation 2

Generation 5

Generation 11



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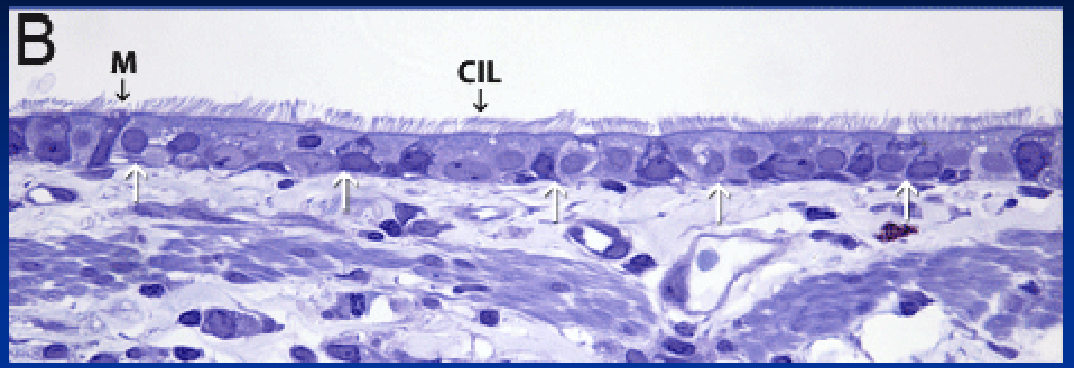
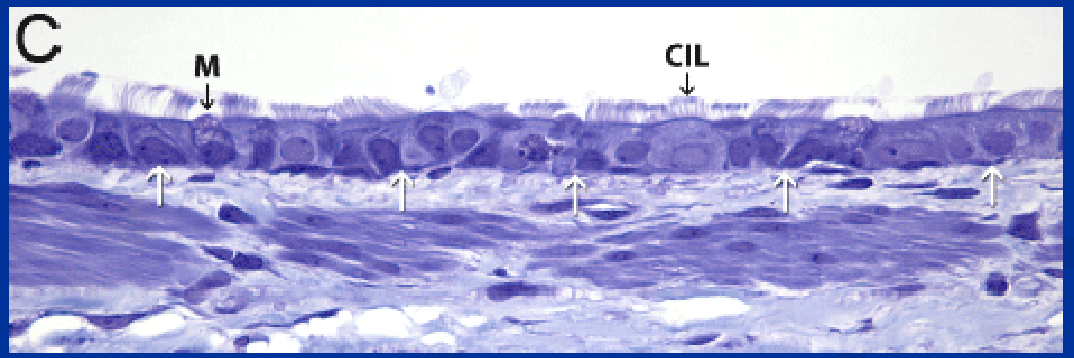
Uncoated Carbon Particles – 24 hours post insufflation

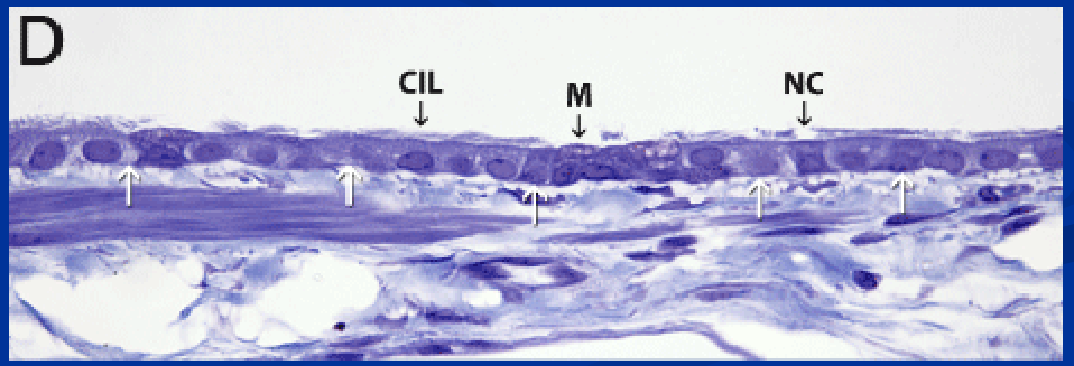
Generation 2

Generation 5

Generation 11







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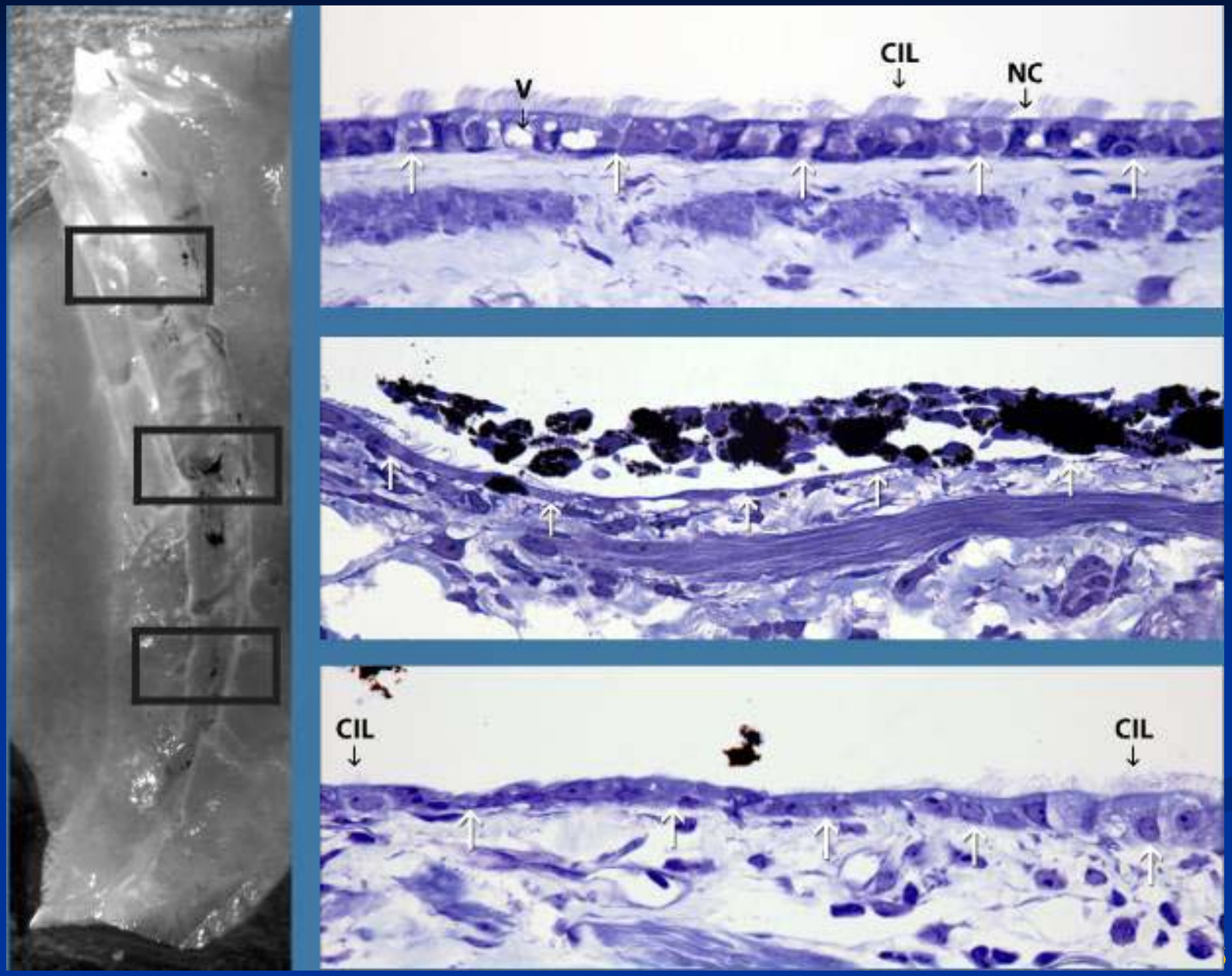
10% 1-NN Carbon Particles – 24 hours post insufflation

Generation 2

Generation 5

Generation 11



1. Pulmonary Metabolic Response

Specific Aim 2

- Compare the pulmonary cytotoxic response of a PAH adhered to a carbon particle with a PAH adhered to a carbon particle containing a transitional metal in postnatal and adult rats

Approach

- **Synthetic particle: carbon + PAH + transitional metal (*iron*)**
- **Exposure: Single intratracheal insufflation: 7-day and adult rats**
- **Evaluation: Early and late timepoints**
  - initial epithelial injury
  - particle clearance
  - site-specific proliferation
  - gene expression profiles
  - site-specific oxidative stress

1. Pulmonary Metabolic Response

Specific Aim 3

- Compare the pulmonary cytotoxic response of a PAH adhered to a carbon particle containing a transitional metal in postnatal and adult rats with and without oxidant stress exposure.

Approach

- **Pre-exposure: 7-day and adult rats to 90 days ozone**
- **Exposure: Single intratracheal insufflation: 7-day and adult rats**
- **Synthetic particle: carbon + PAH + transitional metal (*iron)***
- **Evaluation: Early and late timepoints**
  - initial epithelial injury
  - particle clearance
  - site-specific proliferation
  - gene expression profiles
  - site-specific oxidative stress

1. Pulmonary Metabolic Response

Specific Aim 4

- Compare the pulmonary cytotoxic response of seasonal urban and environmental particulate matter in postnatal and adult rats.

  Approach

  - Environmental Samples (collected in SJV Core)
  - Exposure: *In vitro* (tracheal explants)
  - Evaluation:

    - epithelial permeability/injury
    - gene expression profiles

2. Endothelial Cell Responses to PM -- in *vitro* and in *vivo*

Dennis Wilson and Jack Rutledge

Prior Studies

- Associations between episodes of particulate matter (PM) air pollution and hospital admissions for cardiopulmonary disease are documented worldwide
- Estimates suggest 70% of the increase in cardiac deaths is due to myocardial infarction
- Recent studies emphasize the potential importance of systemic circulation of the ultrafine particulates in polluted ambient air

Project objective

- Determine the effects of ultrafine particulates on endothelial and vascular inflammatory responses
- Evaluate the potential association between atherosclerotic vascular disease and circulating PM

2. Endothelial Cell Responses to PM

Hypothesis

- *Systemic effects on the cardiovascular system are associated with endothelial cell responses leading to activation of inflammatory or clotting cascades. Circulating PM selectively accumulates in regions of the vasculature with endothelial compromise and stimulates the progression of pre-existing vascular disease.*

2. Endothelial Cell Responses to PM

Specific Aim 1

- Characterize human endothelial cell culture responses to direct CAPs exposure.

  Approaches

  - Microarray analysis of PM exposed human endothelium
  - RT-PCR quantitation of Target genes associated with Inflammation
  - Ca^{++} release responses to PM
  - Examine nuclear translocation of second messengers

Specific Aim 2

- Determine the effects of direct PM exposure on permeability and inflammatory cell adhesion in vessels

  Approaches

  - Endothelial monolayer permeability responses to PM
  - Monocyte adhesion responses to PM

2. Endothelial Cell Responses to PM

- **Specific Aim 3**

Compare nature and location of endothelial cell responses in vessels of CAPs exposed mice.

Approaches

- Immunohistochemistry for pro-inflammatory protein expression in CAPs exposed mice
- Laser capture microdissection of arteries with RT-PCR of response genes identified in Aim 1

- **Specific Aim 4**

Determine the effects of CAPs exposure on the progression of preexisting vascular disease in apolipoprotein E (ApoE -/-) deficient mice.

Approaches

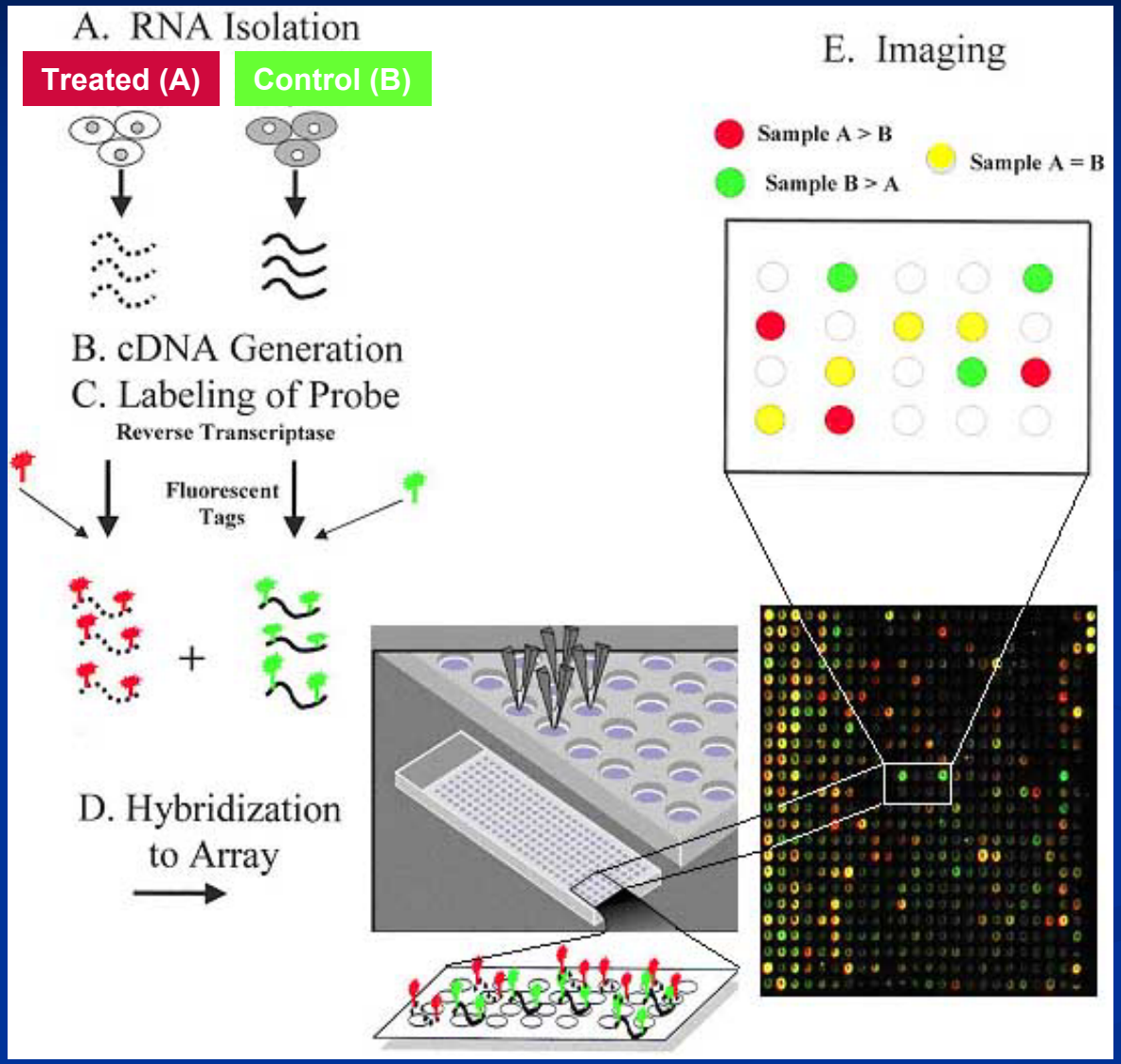
- Monocyte adhesion in isolated carotid arteries from CAPs exposed normal and ApoE -/- mice
- Laser capture microdissection of atheromatous lesions from CAPs exposed ApoE -/- mice

Experimental Approaches

Endothelial Cell Responses to PM

- Signal Transduction in response to PM
- Gene responses to PM
  - In vitro by microarray
  - In vivo by LCM and RT-PCR
- Functional Consequences of PM exposure
  - Vascular permeability and inflammation
  - Monocyte adhesion

Microarray Analysis of Gene Expression



Control Samples:

Green Dye **Cy3**

Experimental Samples:

Red Dye – **Cy5**

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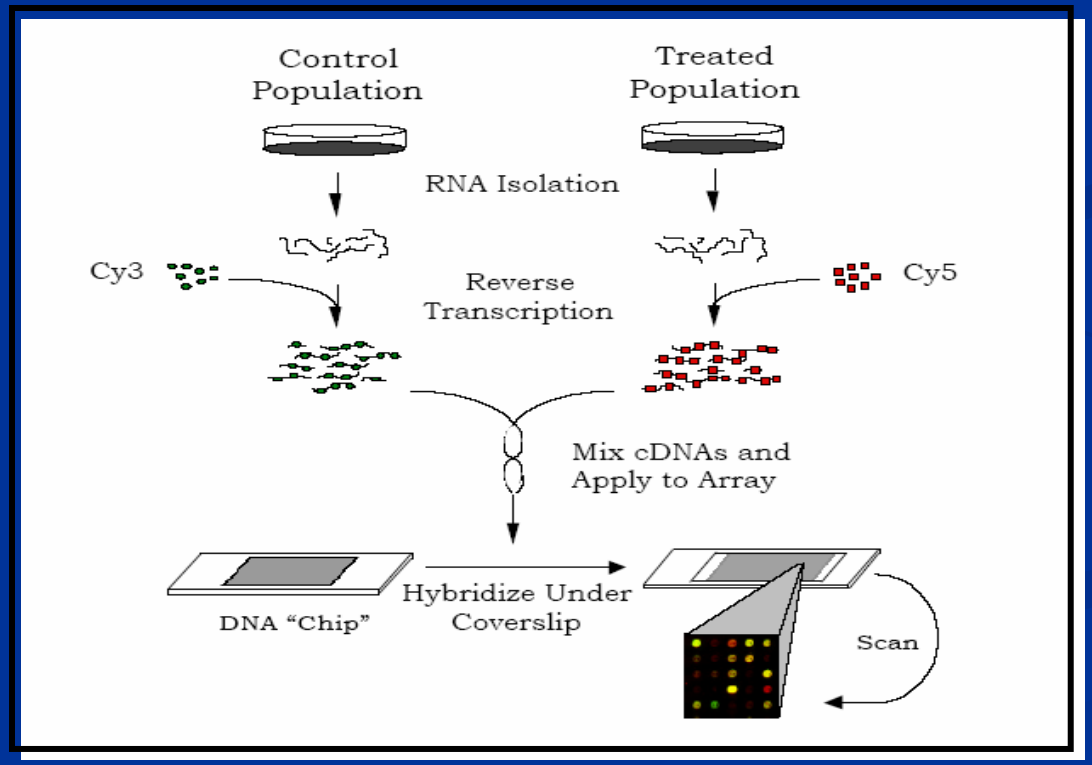
Comparative Gene Responses to CAPs

- Human Aortic Endothelial Cells
- Control vs. Regional CAPs
- Comparison Between Groups by Cluster Analysis
  - Functional Gene Groups (Inflammation, Proliferation, Cell Death, Thrombosis)



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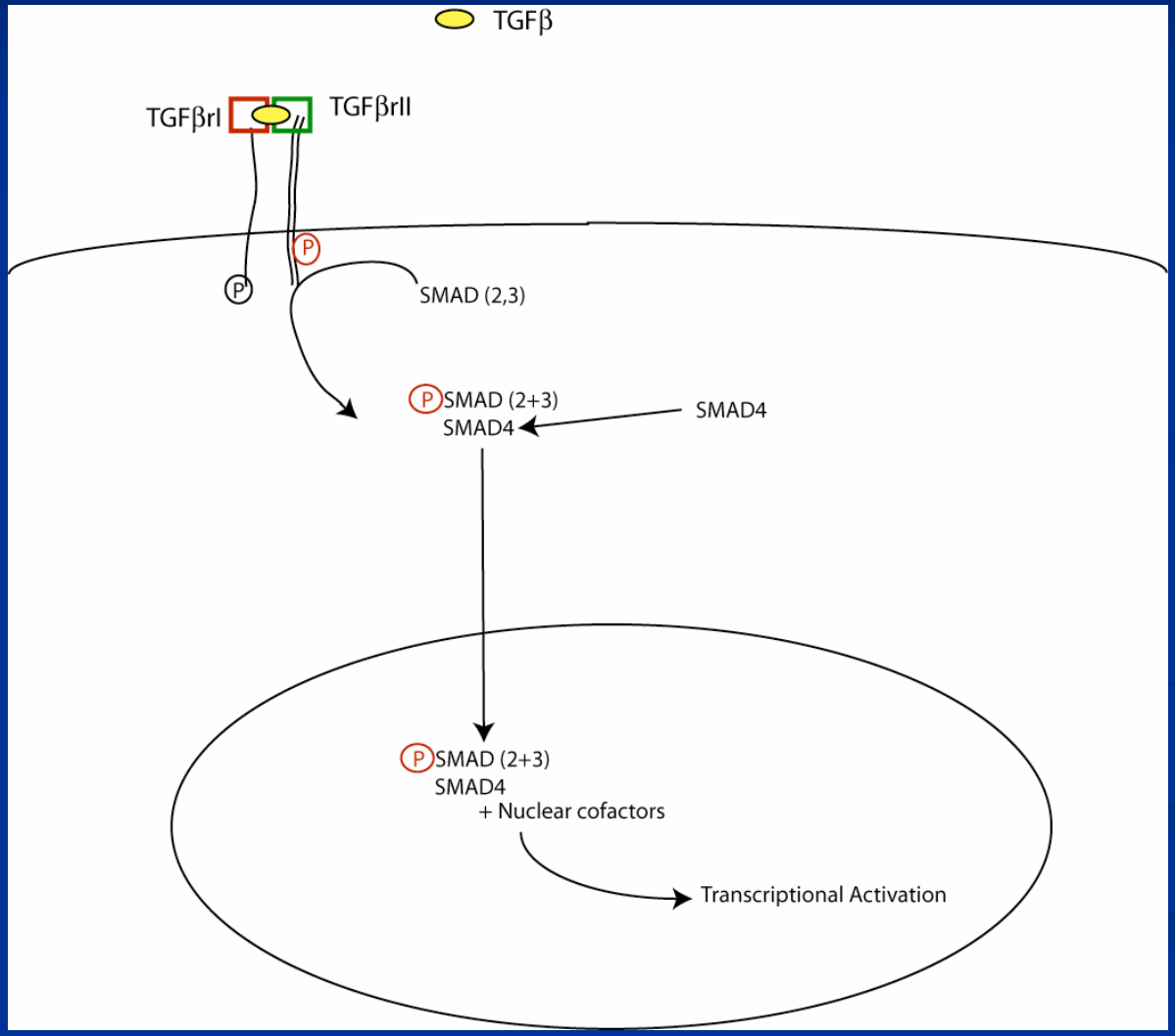
TGF-β/Smad Signaling and Endothelial Responses



proliferation, migration, differentiation and apoptosis

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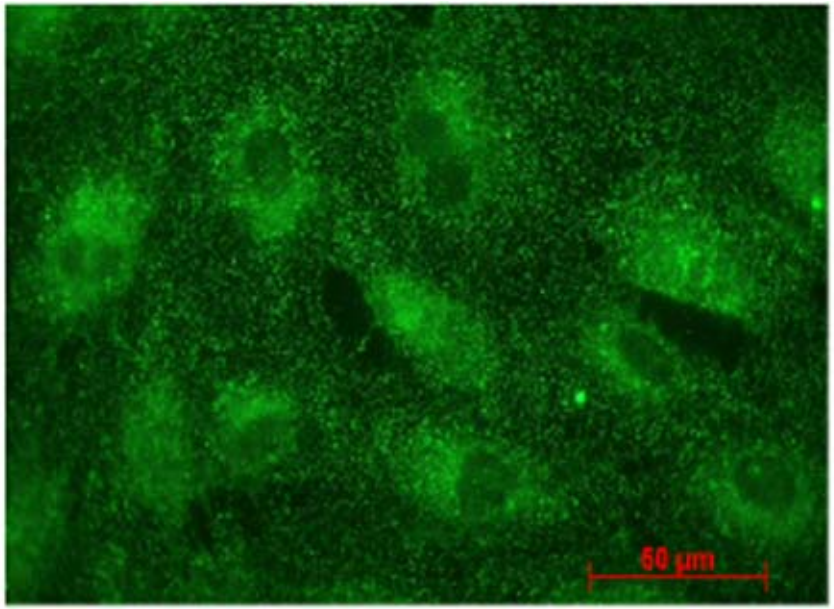
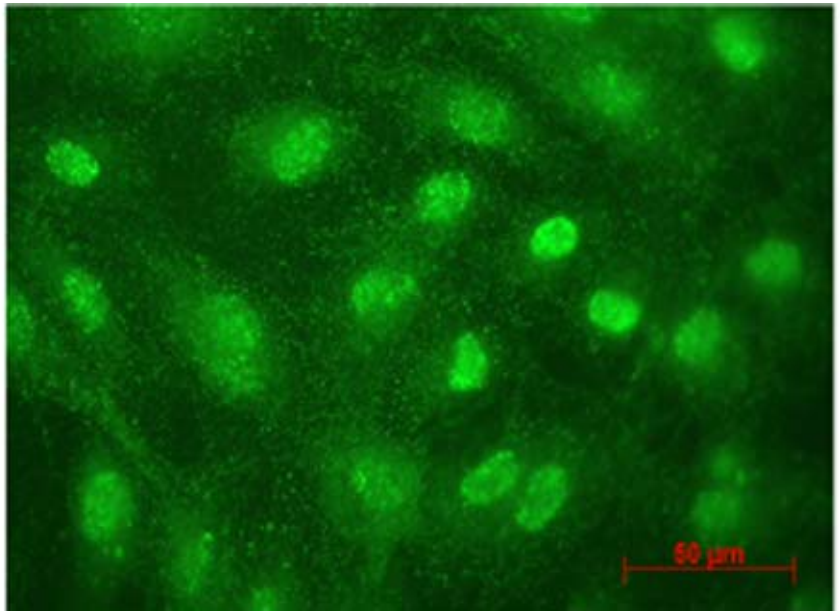
SMAD Signaling in HPAEC

60 min post signal





3. Inhalation Responses to SJV Aerosol

Kent Pinkerton, Mike Kleeman, Ann Bonham

Prior Studies

- Preliminary epidemiological evidence suggests cardiac mortality in SJV is strongly correlated with PM10
- Characterized spatial and temporal variability of size and composition of airborne particles in the SJV

Project objective

- Determine how variation in particle concentration, size and/or composition affects heart rate variability and oxidative stress in mice exposed to concentrated airborne particles at urban and rural location in SJV during the summer and winter
- Chemical "fingerprints" used to determine ambient particulate sources correlated with severe health outcomes

3. Inhalation Responses to SJV Aerosol

Hypothesis

- *Size and composition distribution of airborne particles affect health outcomes through different*
  - *mechanisms of oxidative stress, and*
  - *impacts on heart rate variability*

3. Inhalation Responses to SJV Aerosol

Specific Aim 1

- Test whether differences in particle size and composition that occur naturally in the SJV as a function of location and season have an effect on health outcomes.

Approach

- **Expose mice to concentrated airborne particles at an urban and rural location in the SJV during summer and winter. Monitor heart rate variability and markers for oxidative stress. Collect samples of airborne particles at the same time to correlate particle size and composition with health outcomes.**

3. Inhalation Responses to SJV Aerosol

Specific Aim 2

- Determine the source(s) of particles used in exposure experiments in specific Aim 1.

Approach

- **Use chemical "fingerprints" to determine the source of particles collected in Specific Aim 1 that correlate with severe health outcomes.**

3. Inhalation Responses to SJV Aerosol

Specific Aim 3

- Test whether exposure to freshly emitted particles from individual sources causes same health outcomes as exposure to a mixture of aged particles.

Approach

- **Expose mice to freshly emitted particles from sources dominating exposure during Specific Aim 1. Heart rate variability and markers for oxidative stress will be monitored. Collect samples of airborne particles at the same time to enable correlation of particle size and composition with health outcomes.**

3. Inhalation Responses to SJV Aerosol

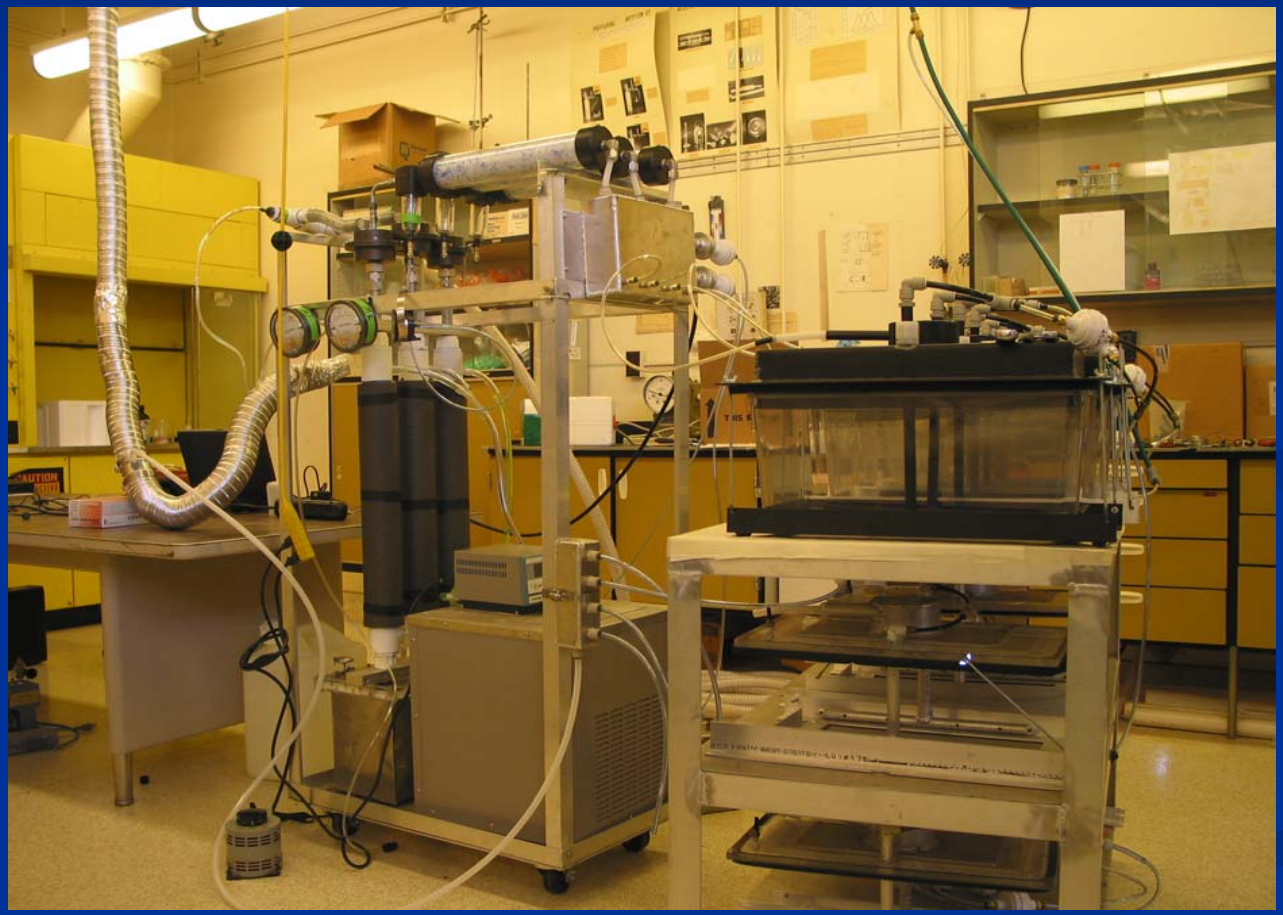
Specific Aim 4

- Identify the specific particle size and composition that cause negative health outcomes.

Approach

- **Plausible mechanisms relating negative health outcomes to particle size and composition will be tested using laboratory particles that mimic features of particles released directly from sources.**

VACES Particulate Matter Concentrator and Biosampler



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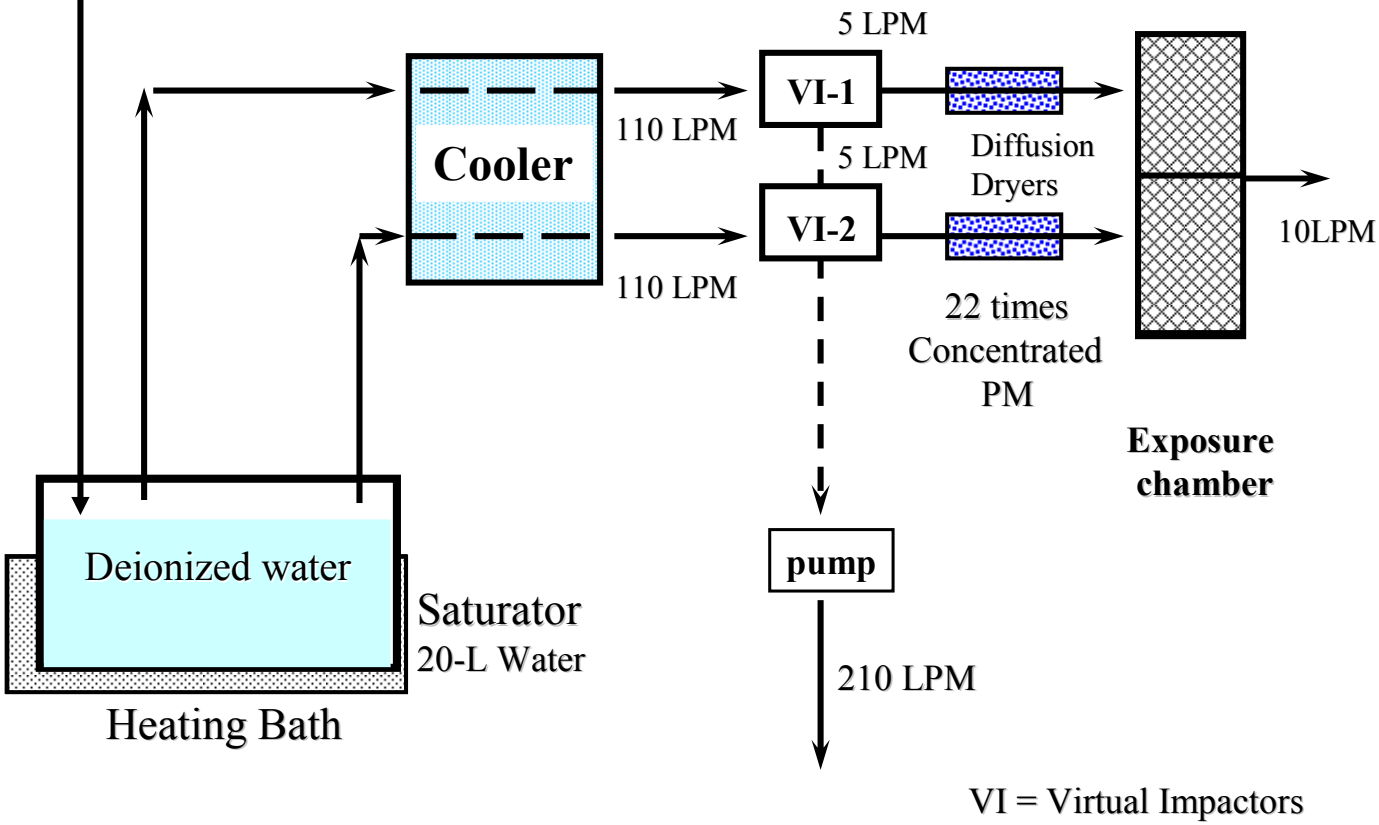
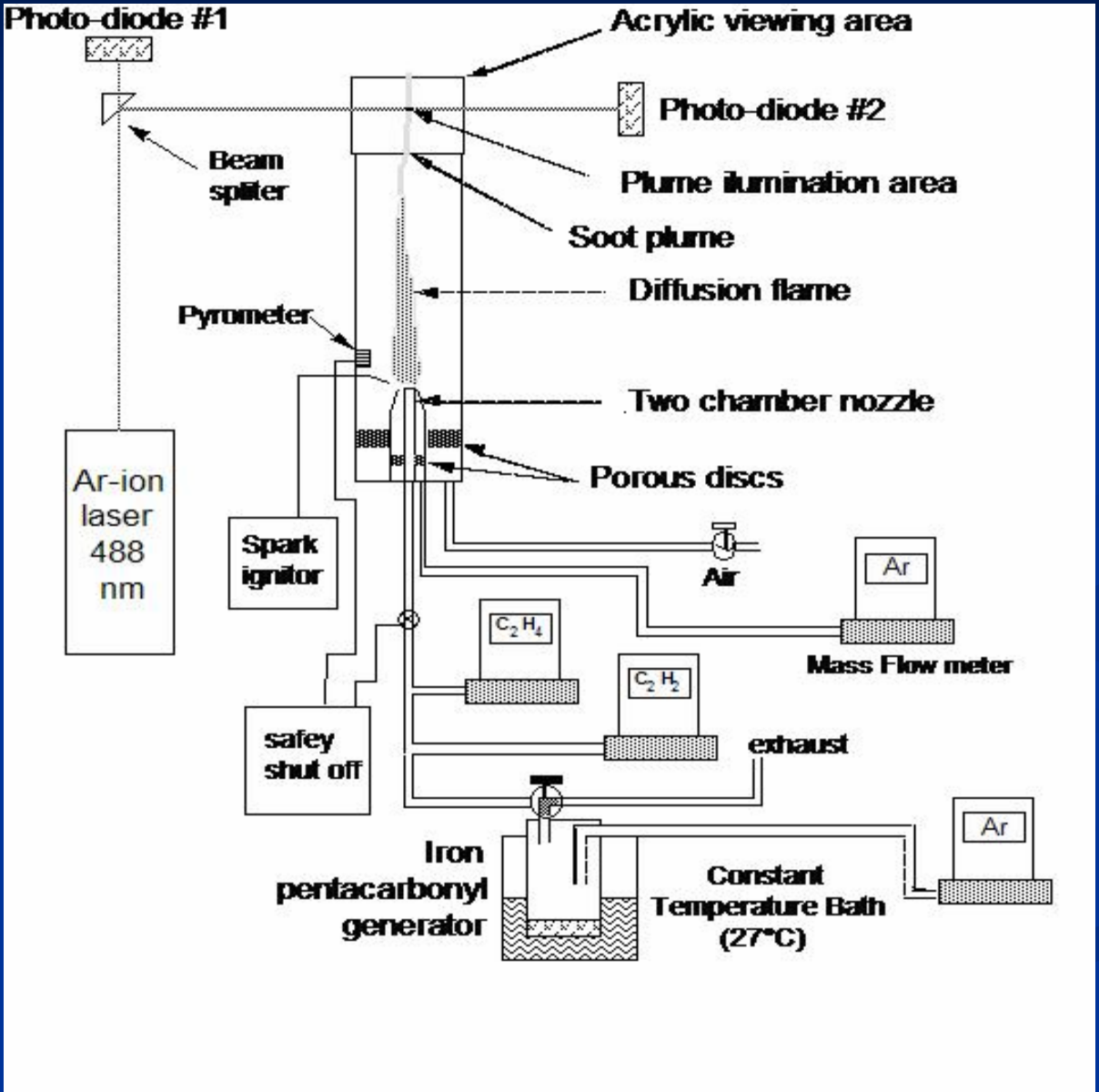
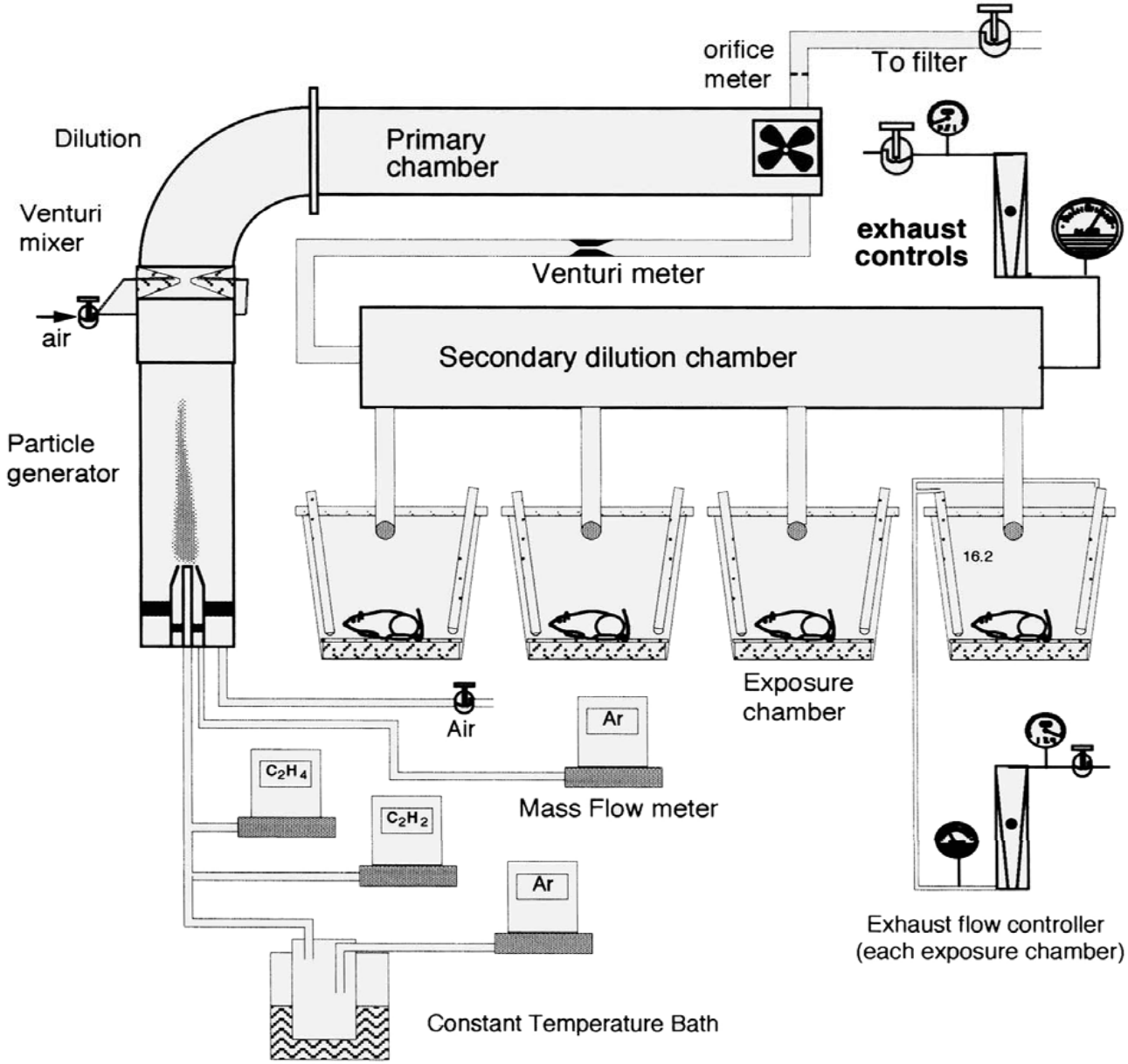
PM Concentrator System



Diffusion Flame Apparatus



Particle Generation/Inhalation System



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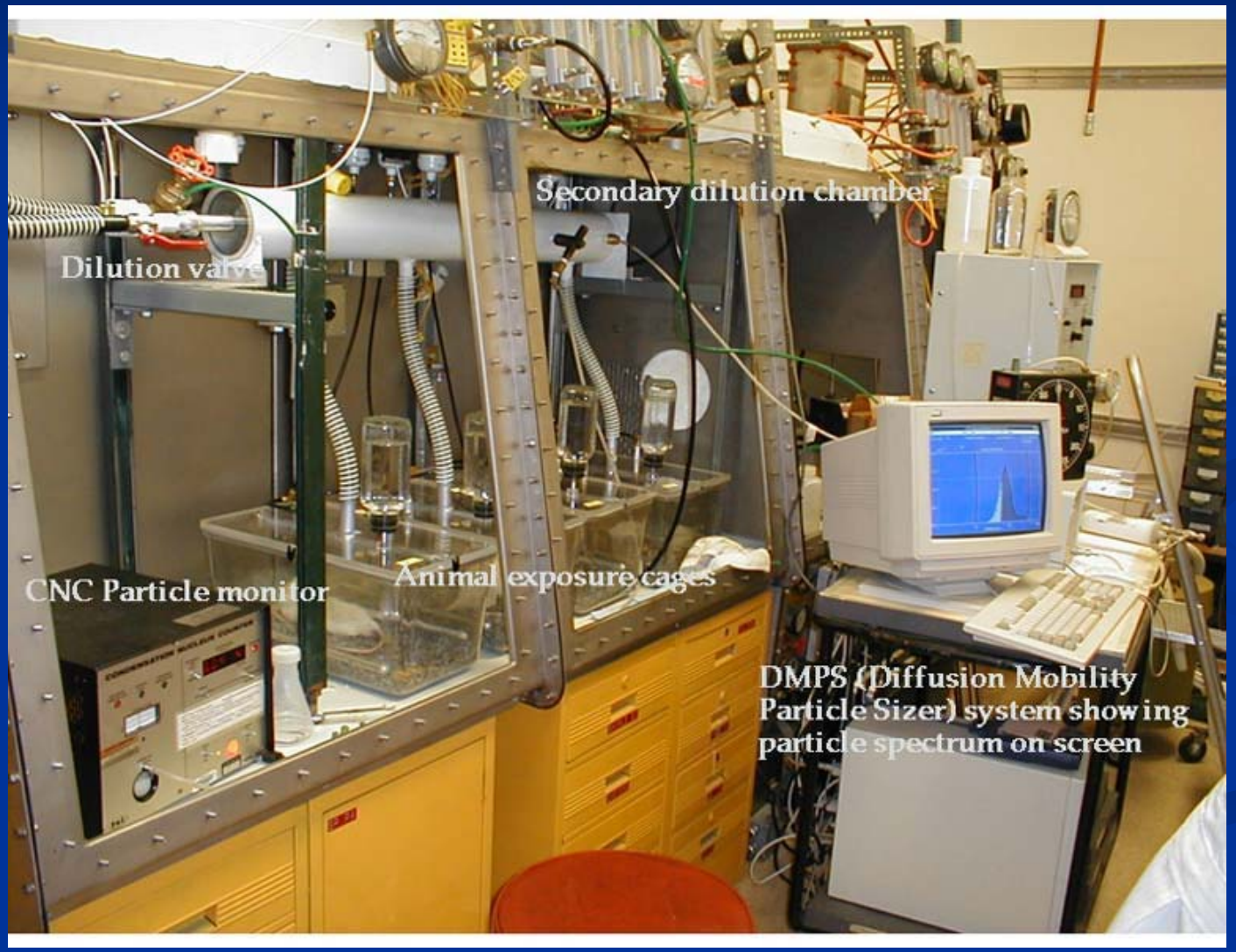
Deriving Aerosols from Archived PM for Inhalation Studies



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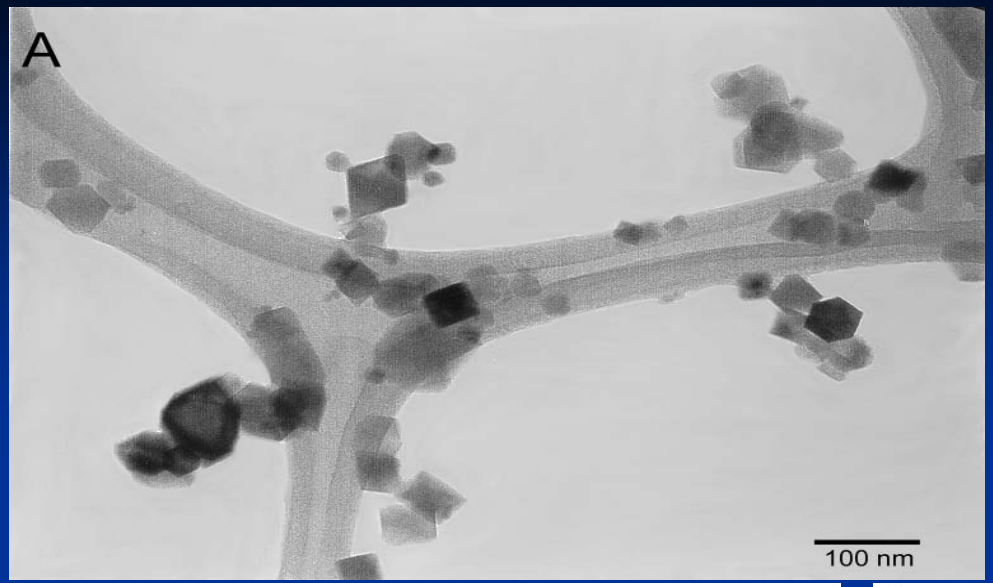
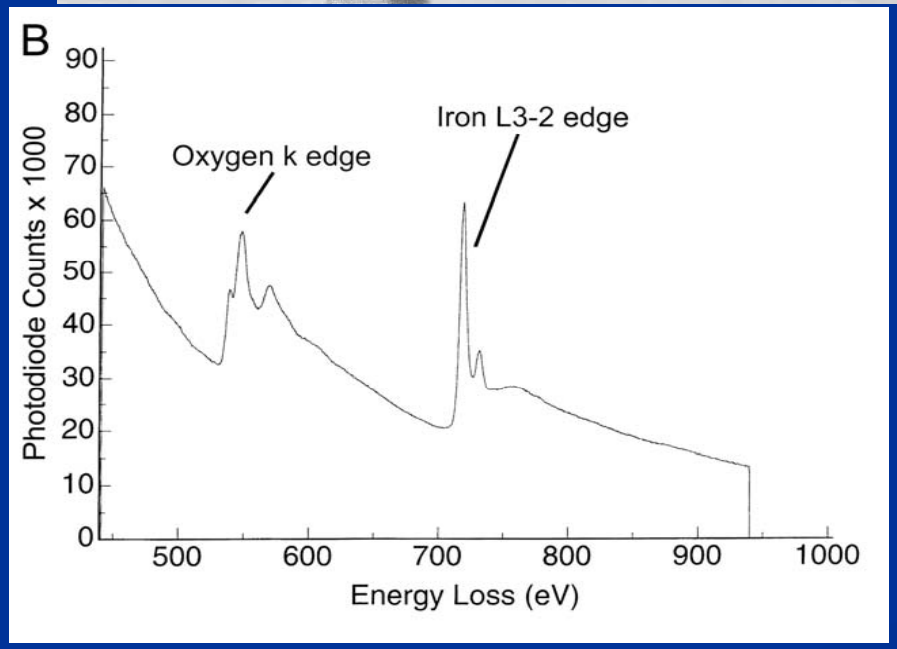
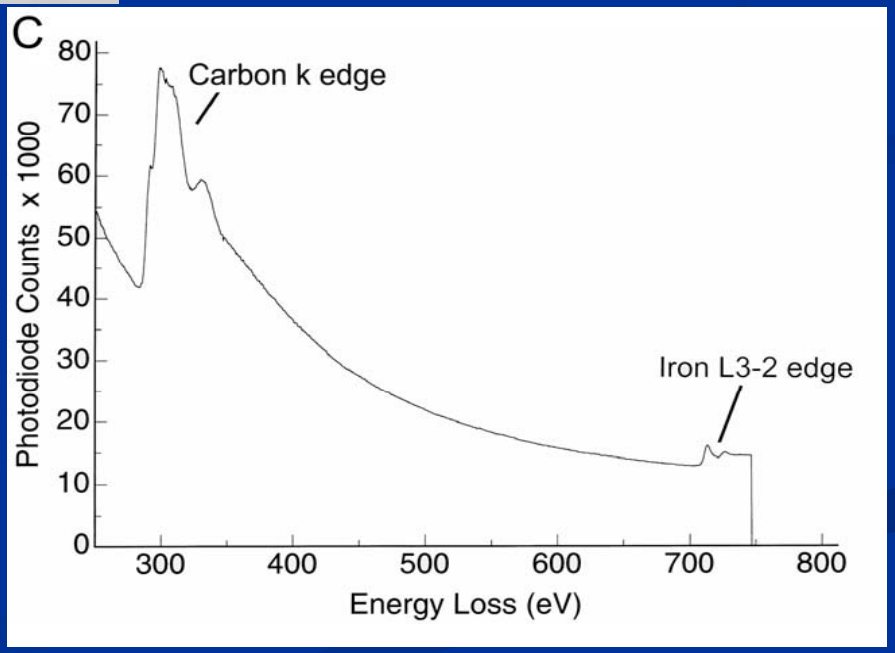
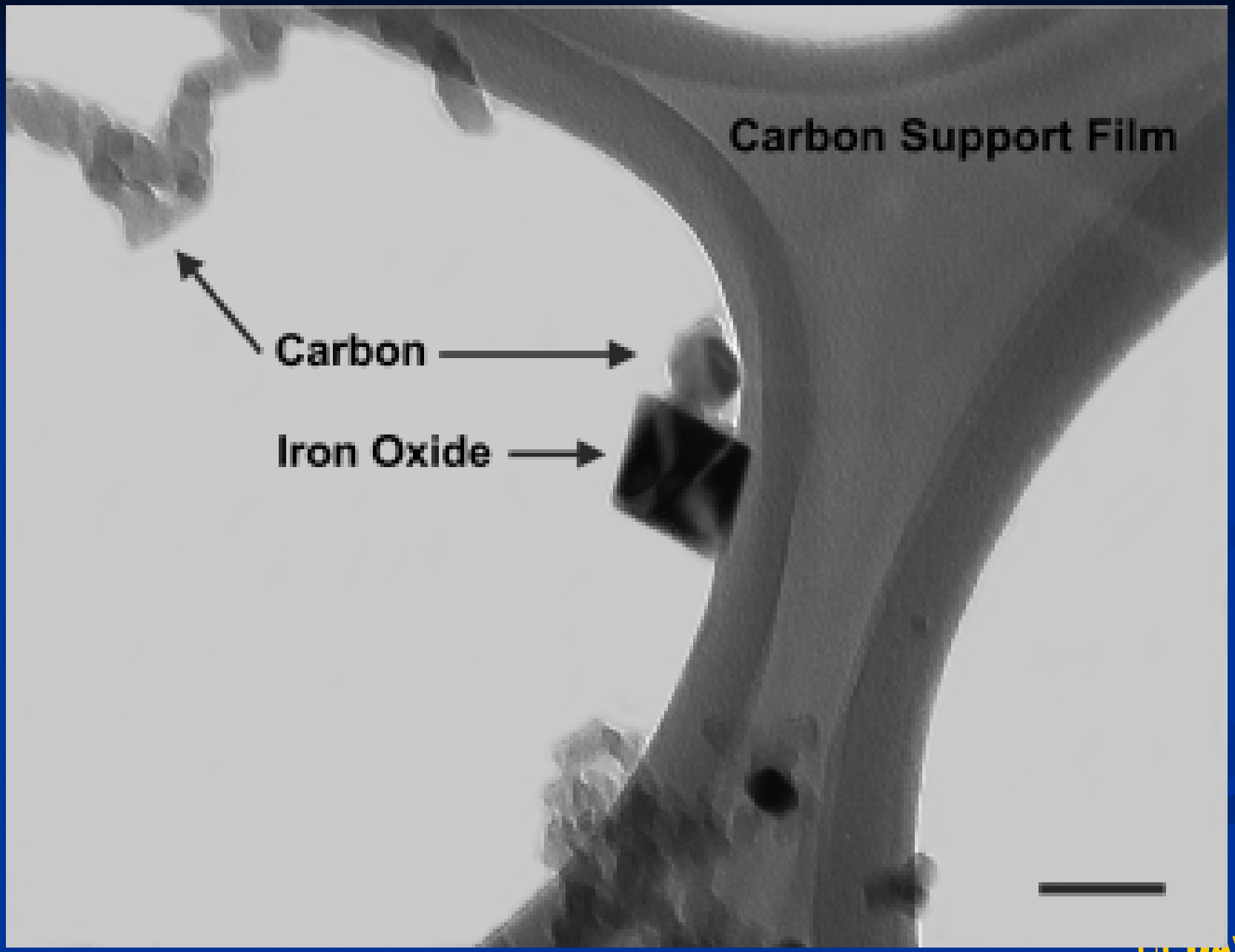
Iron Particles and EELS analysis








Carbon Support Film
Carbon
Iron Oxide

Dust Generator

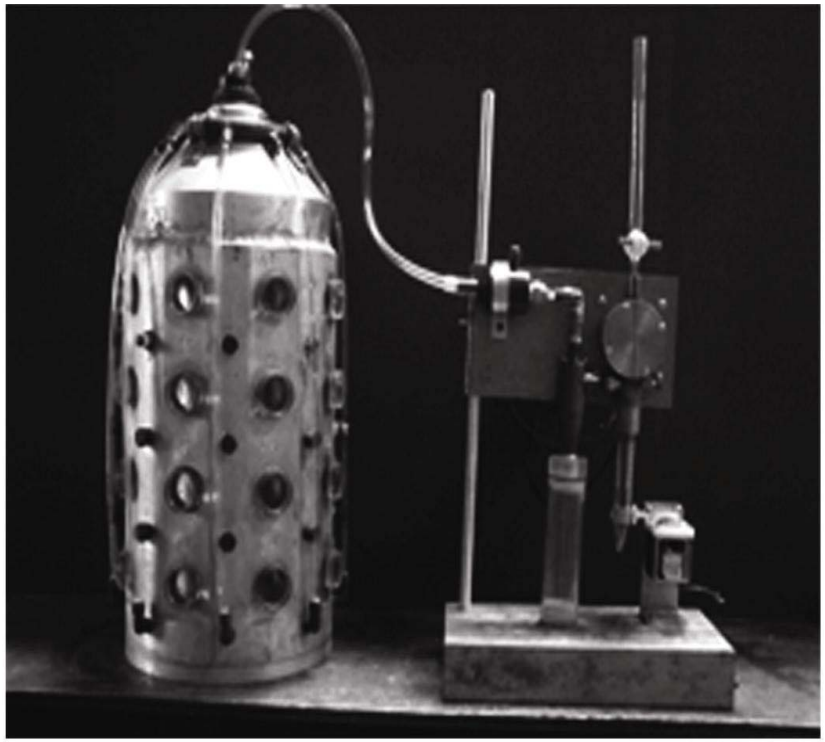


Figure 1. The assembled dry powder aerosol generator (right) connected to a nose-only exposure chamber (left).

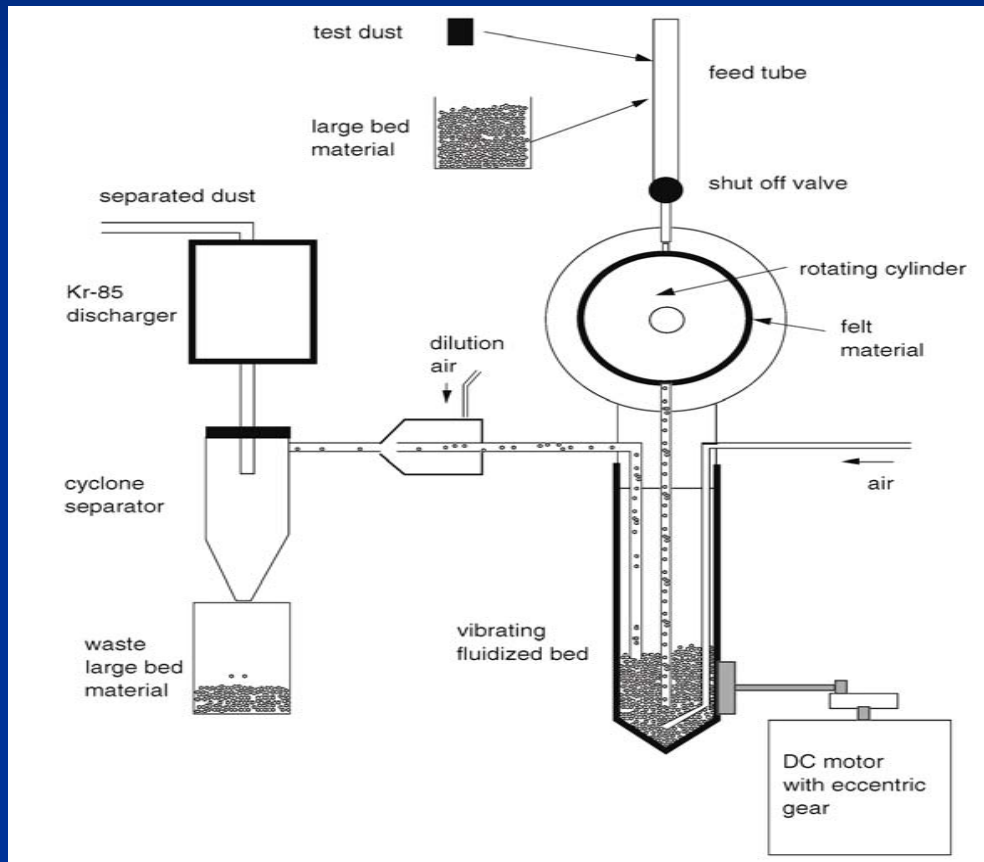


Figure 2. Schematic flow diagram of the aerosol generator system.

Appl Opt, **41**, 5148 (2002)

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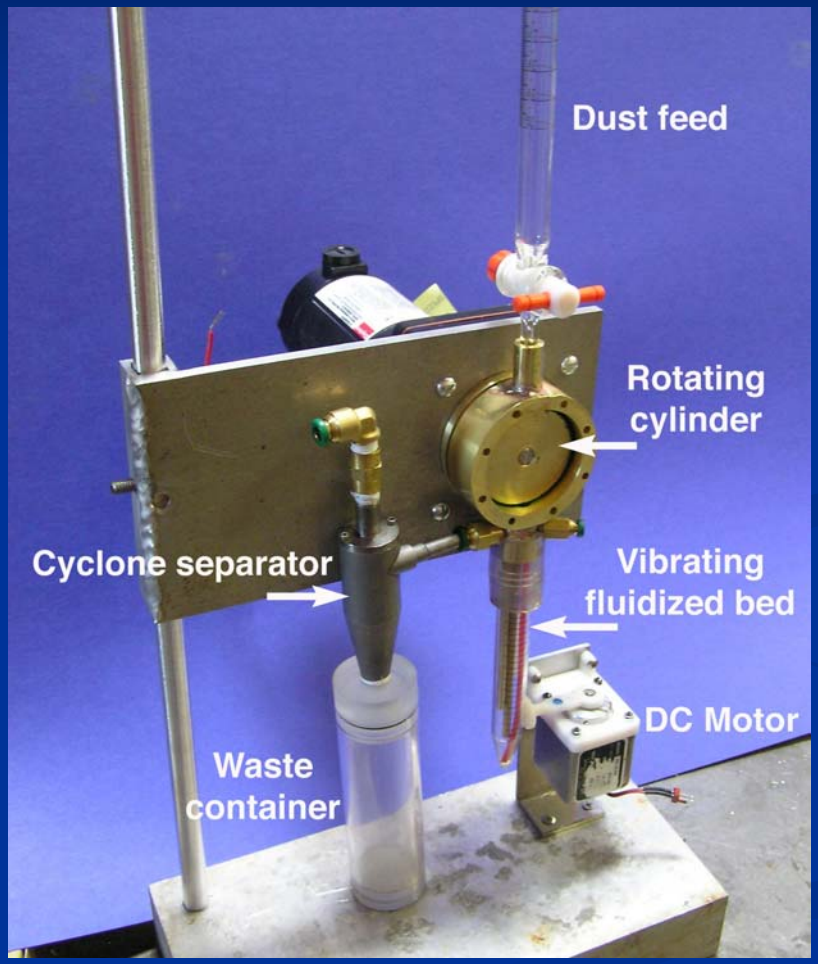
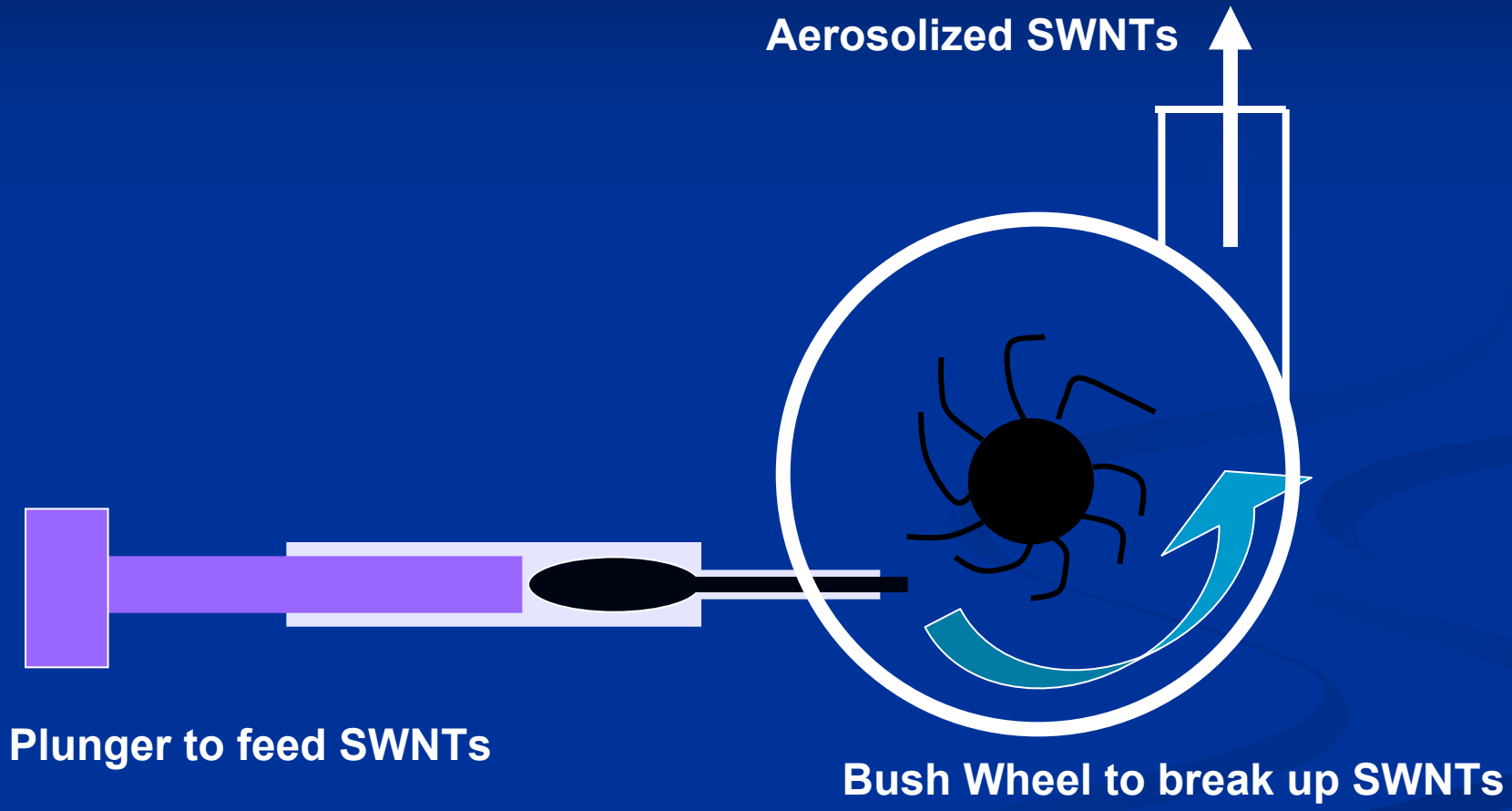
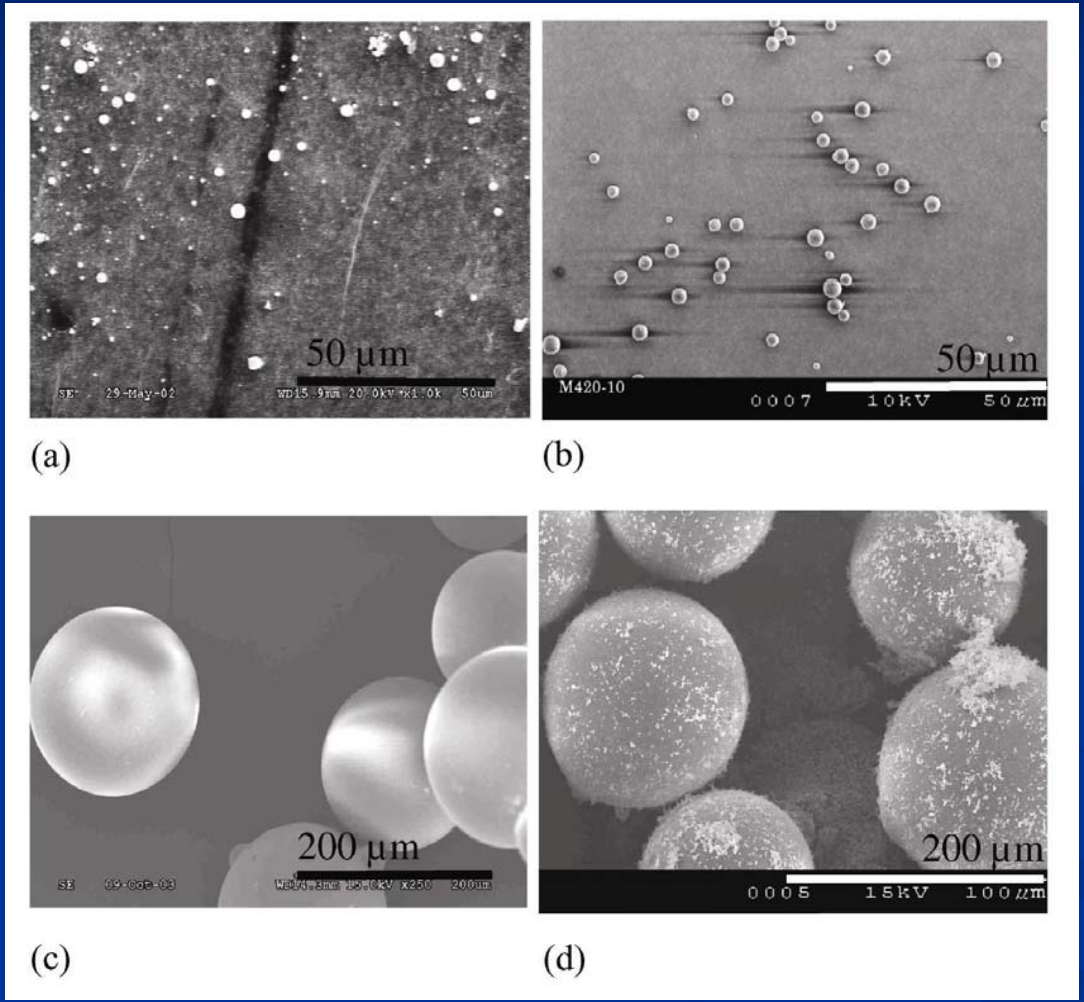
Aerosol particle generator



Bush-Wheel SWNT Aerosolizer



Silica Bead Carriers/CB



Some Carbon blacks stick to the surface of the glass beads.

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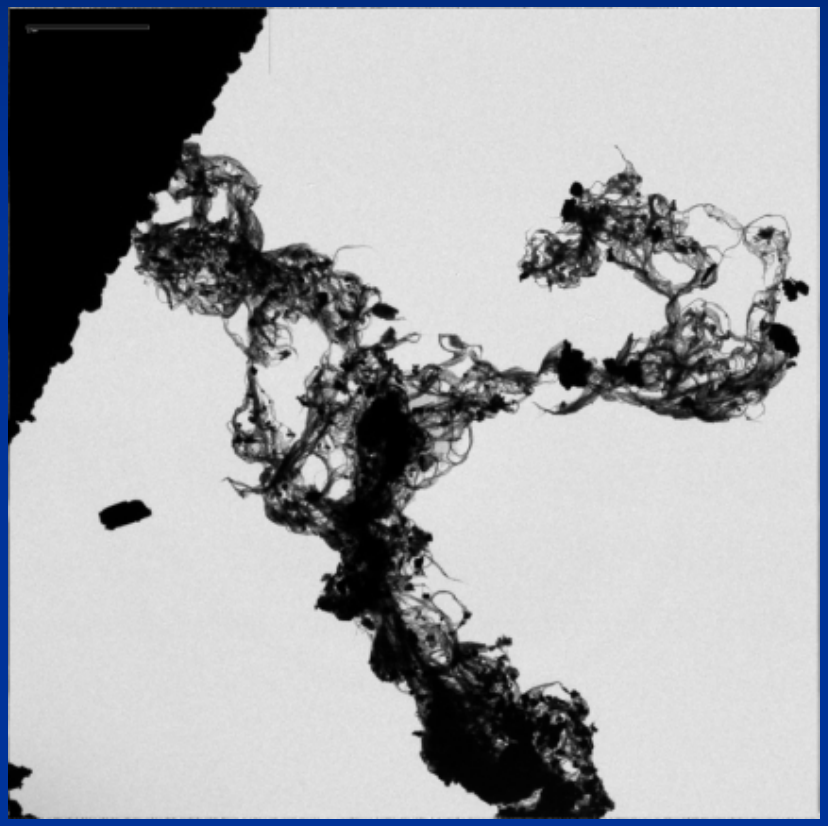
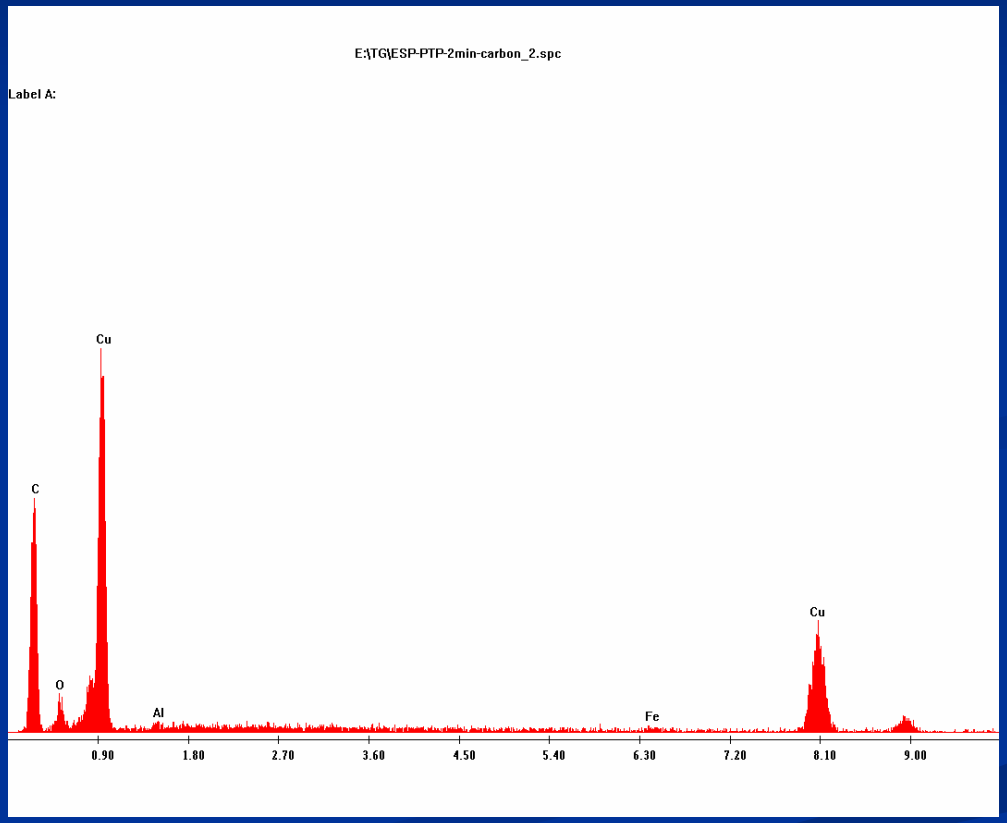
Elemental Analysis of PM





1-2 m size irregular shaped SWNTs aerosols are found and confirmed with EDS

Black flakes are Al chips

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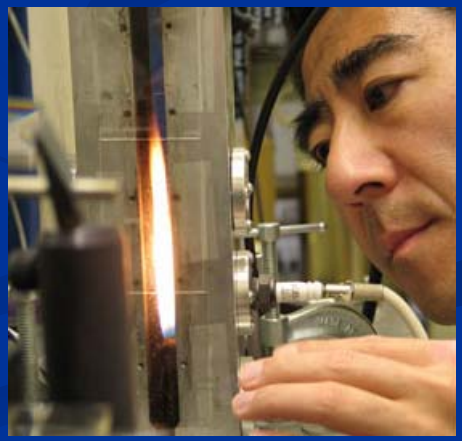
4. Transport Mechanisms and Systemic Fate of Inspired Ultrafine Particles

Dennis Wilson, Angelique Louie, Ian Kennedy, Michelle Fanucchi, Alan Buckpitt

Prior Studies

- Recent evidence demonstrates that ultrafine particles diffuse rapidly from the lungs into systemic circulation
- Mechanisms of ultrafine transport to systemic circulation and tissues remain largely uncharacterized



Project objective

- Determine the effects of size and charge on the time course, distribution and mechanisms of accumulation of PM in circulation and tissues of animals with normal and altered lung structure

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4. Transport Mechanisms and Systemic Fate of Inspired Ultrafine Particles

Hypothesis

- *Trans-epithelial movement of inhaled PM results in accumulation in target organs based on endothelial cell facilitated transport.*

4. Transport Mechanisms and Systemic Fate of Inspired Ultrafine Particles

Specific Aim 1

- Characterize time course and distribution of circulating particulates *in vivo*

  Approaches

  - Kinetics of Indium labeled particulates after insufflation
  - Distribution within components of blood
  - Effect of size and charge on absorption and distribution

Specific Aim 2

- To compare anatomic site of particulate accumulation in tissues with organ distribution as determined by microimaging techniques

  Approaches

  - PET of dextran coated iron oxide particles conjugated to Cu^{64} and labeled with fluorochrome
  - Quantitative distribution of fluorescent particles after inhalation exposure using plastic embedded tissues from target organs
  - Comparison with rats given lifetime O_3 exposures

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Specific Aim 3

- To evaluate potential mechanisms of PM transport across epithelial and endothelial barriers.

  Approaches

  - Confocal and deconvolution microscopy of HAEC and BEAS2b cells exposed to fluorescent particles of varying size and charge
  - Determine effects of inhibitors of specific pathways of endothelial facilitated transport

Specific Aim 4

- To characterize the dynamics of interaction between particulates and airways and arterial walls.

  Approaches

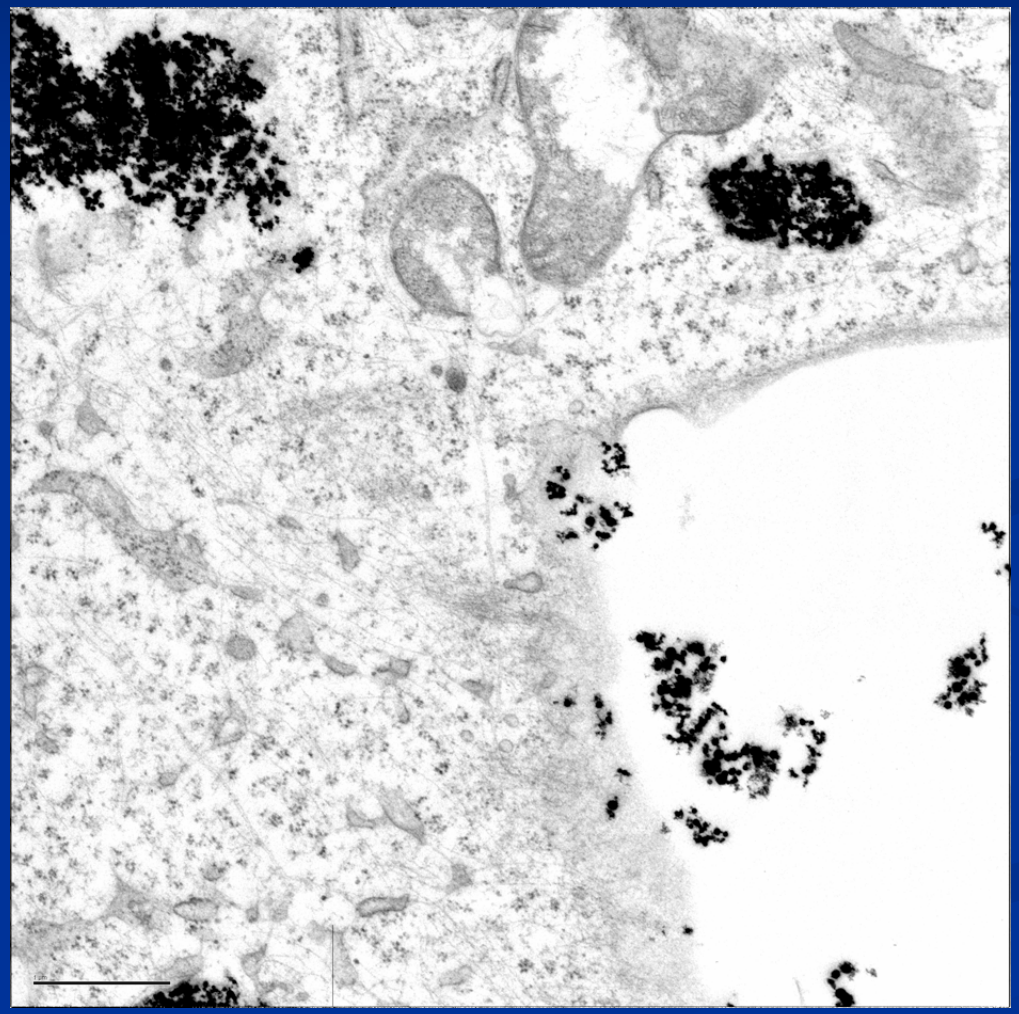
  - Fluorescent PM uptake in isolated tracheas and carotid arteries from normal mice
  - Tissue localization in plastic embedded sections by confocal microscopy

Experimental Approaches

Transport Mechanisms and Systemic Fate of Inspired Ultrafine Particles

- Mechanisms of particle transport through cells
- Microimaging of radiolabeled particles in laboratory rodents
- Correlative localization of fluorescence tagged particles in tissues

Lung tissue exposed to 50 ug of Iron (Fe)



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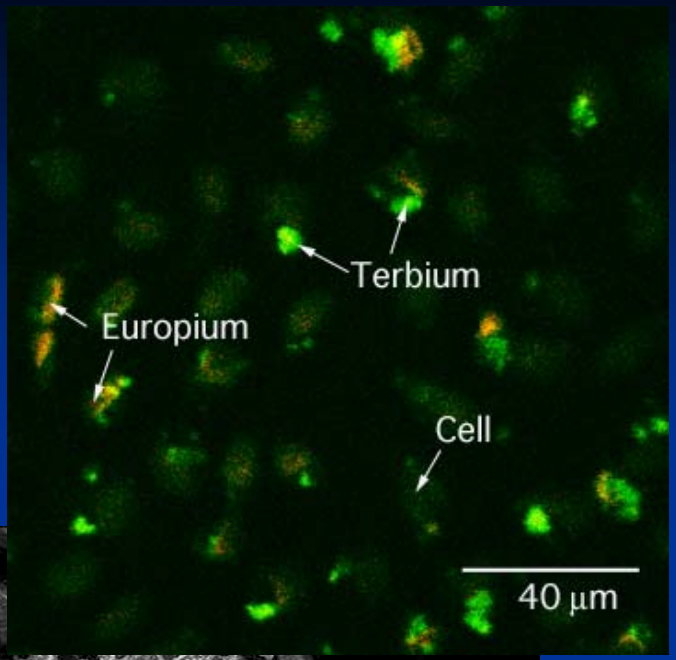
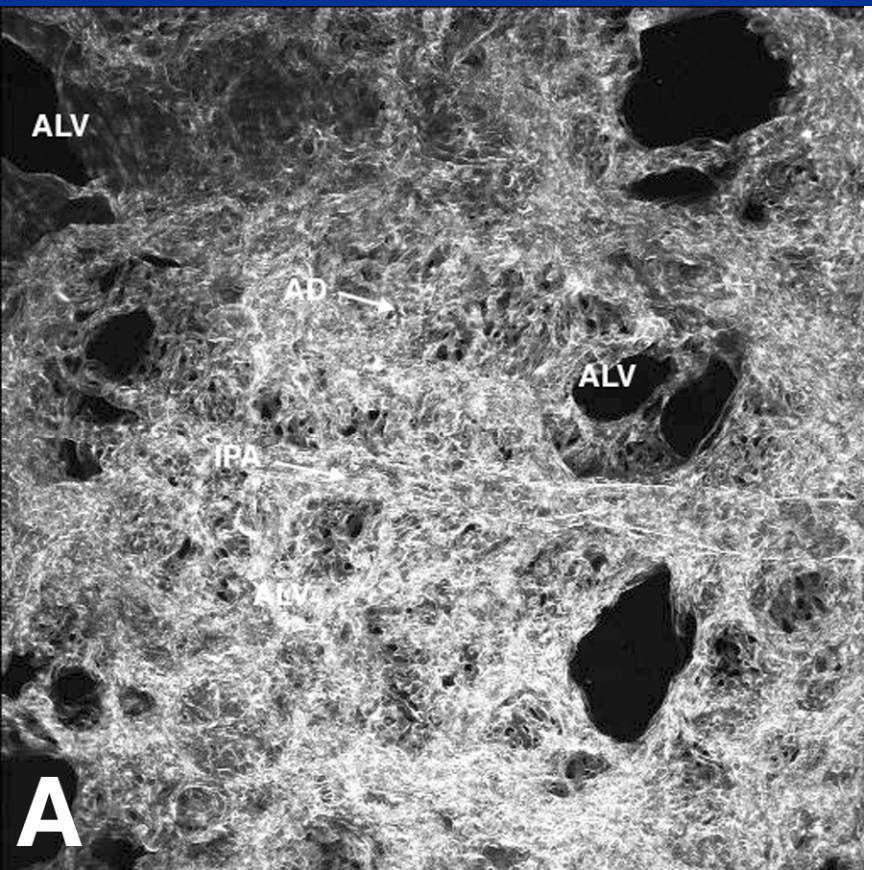
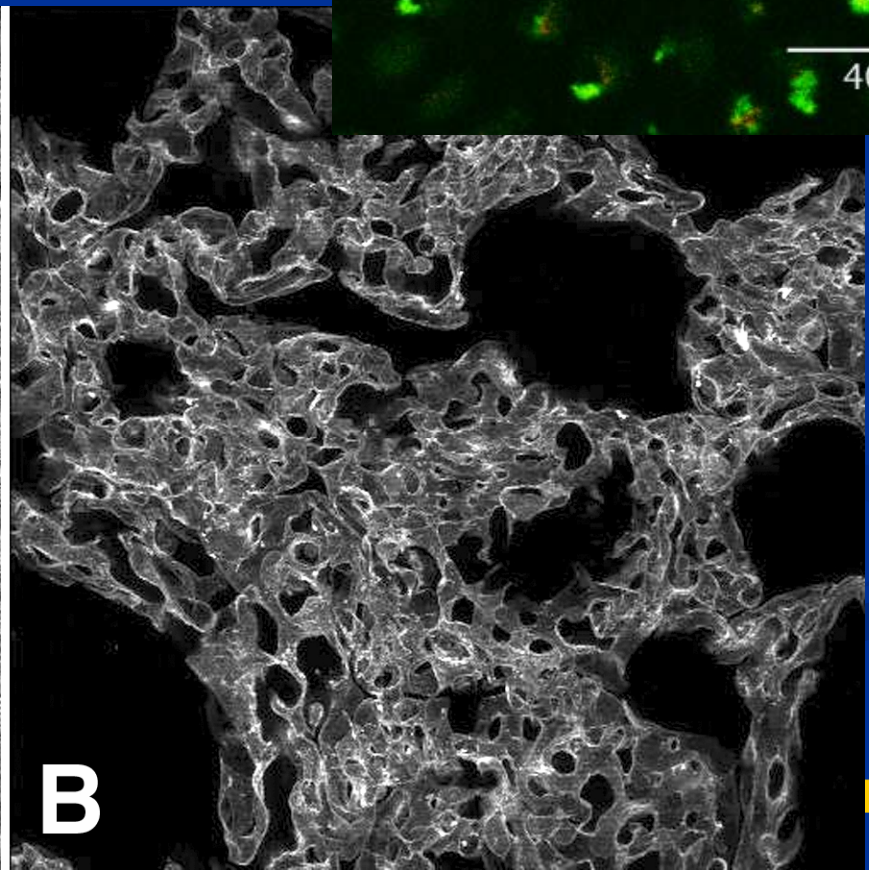
MicroPET Imaging

Special Microscopic Techniques -- used to find ultrafine particles in tissues







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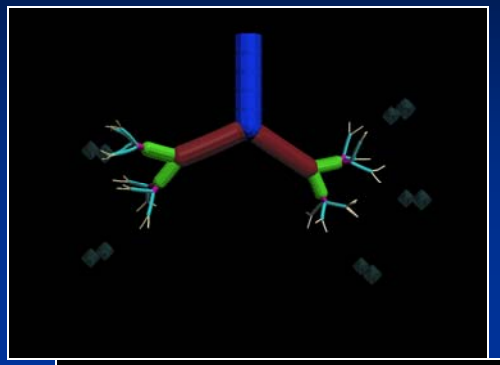
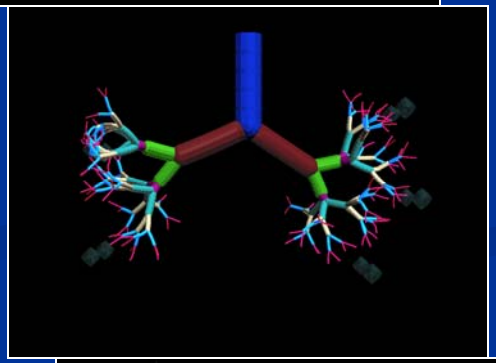
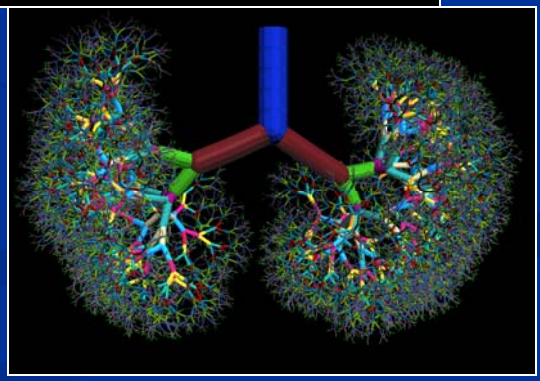
5. Developmental Response and Particle Deposition

Tony Wexler and Charlie Plopper







Prior Studies

- Epidemiological evidence suggests that children exposed to air pollution develop impaired lungs
- Previously observed alterations in development of lung architecture in postnatal monkeys exposed to ozone

Project objectives

- Quantify the amount and time course of pollutants that lead to lung architectural abnormalities and their functional implications

Children are not “Little Adults”

- Children breathe more air in proportion to body weight
- Children are mouth breathers
- Children spend more time outdoors
- Children have more time to develop environmentally-induced disease with long latency periods

5. Developmental Response and Particle Deposition in the Lung

Hypothesis

- *Exposure of young children to air pollutants during critical windows of postnatal airway development compromises airway growth and alters airway architecture, which:*
  - *diminishes lung function due to the development of non-optimal airway architecture and*
  - *shifts intrapulmonary particle deposition patterns due to altered flow rates and airway geometries.*

5. Developmental Response and Particle Deposition in the Lung

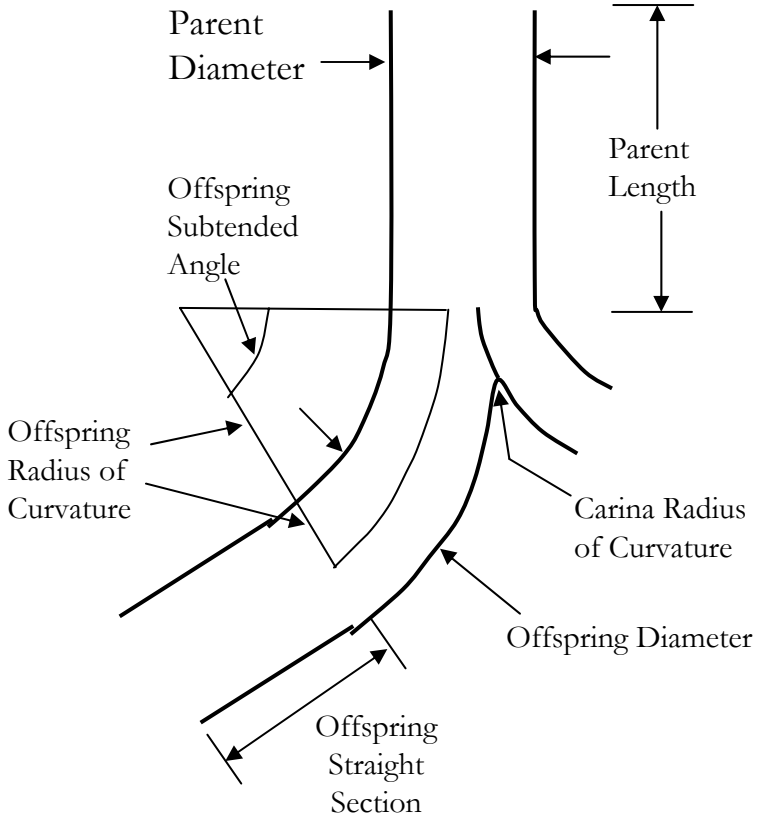
Specific Aim 1:

- Test whether normal pattern of dysanaptic growth of airways in neonates alters development of airway architecture and patterns of airflow from that in adults.

Approach

- Provide neonates with clean, filtered air to breathe during development. Perform lung function tests. Their lungs will be fixed, excised, and casted. Image the casts to reveal the lung architecture, which will be compared at sequential stages of development.

Geometric Model of an Airway Bifurcation



- Model is used to quantify airway architecture parameters from CT voxel data

5. Developmental Response and Particle Deposition in the Lung

Specific Aim 2

- Test whether dysanaptic postnatal growth alters deposition of inhaled particles within the respiratory tract of infants and young children as they grow.

Approach

- Expose neonates and adults to variable-sized particles, and image the deposition pattern. Develop mathematical models of particle deposition to predict these patterns and identify cause for the deposition patterns.

5. Developmental Response and Particle Deposition in the Lung

Specific Aim 3

- Test whether exposure to oxidant air pollutants during critical phases of airway growth compromises postnatal airway growth.

Approach

- Expose neonates to ozone for various time courses during periods of rapid growth. Using approaches in Aim 1, identify lung architecture and compare to normals characterized in Aim 1 to quantify their variation and deviation from the norm.

5. Developmental Response and Particle Deposition in the Lung

Specific Aim 4

- To test whether exposure to particles and ozone during critical phases of airway growth compromises growth to a greater degree than exposure to particles or ozone alone.

Approach

- Expose neonates to a range of particle sizes, compositions, and morphologies, with and without ozone, as a function of stage of development. Identify the architecture and alteration due to exposure using the approaches and normals from Aim 1. Compare these results to those obtained in Aim 3.

5. Developmental Response and Particle Deposition in the Lung

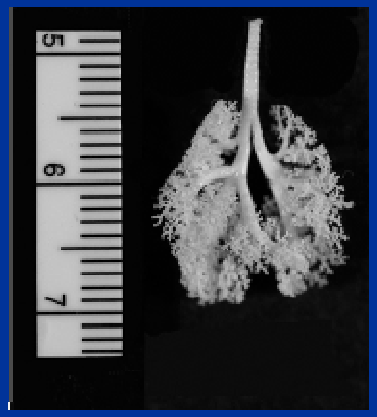
Specific Aim 5

- Test whether compromised airways produce altered patterns of intrapulmonary particle distribution and deposition.

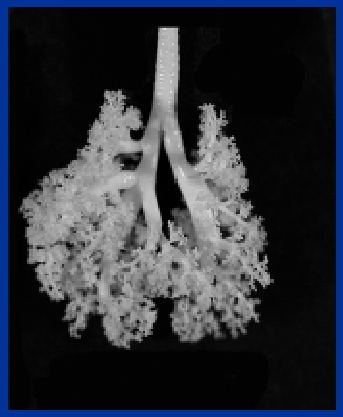
Approach

- Expose normal adults and adults who were pollutant-exposed during development to variable-sized particles. Image the particle deposition pattern. Develop mathematical models of particle deposition to predict deposition patterns and identify the cause for the deposition patterns, similar to Aim 2.

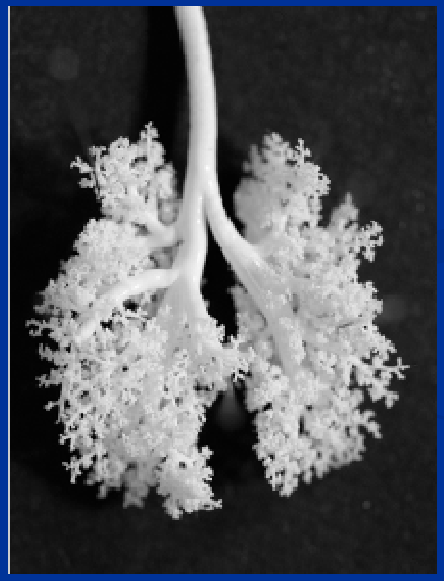
Postnatal Airway Tree Growth



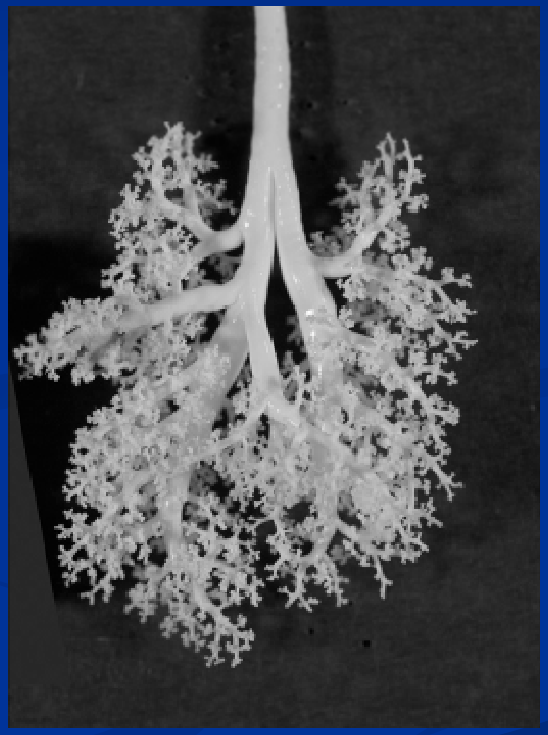
7 Day



14 Day



28 Day



Adult

Projects and Cores

- Projects
  - 1: Pulmonary Metabolic Response
  - 2: Cardiovascular Metabolic Response
  - 3: SJV Aerosol Inhalation Exposure
  - 4: Transport and Fate of Particles
  - 5: Architecture Development
- Cores
  - Animal Exposure
  - Particle Generation, Modification, and Characterization
  - San Joaquin Valley
  - Imaging
  - Bioanalytical

  - Quality Management
  - Administrative

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Thanks

- Questions?